View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Copenhagen University Research Information System

UNIVERSITY OF COPENHAGEN

Introduction

Houe, Hans

Publication date:
2007

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Houe, H. (2007). *Introduction*. Abstract from DubPar seminar - a closing seminar on the project Diagnosis, disease dynamics and intervention: *Salmonella* Dublin and Paratuberculosis CEPROS ll-7, Copenhagen, Denmark.

Download date: 07. apr.. 2020

Proceedings of the DubPar seminar

March 22, 2007
Copenhagen, Denmark

A closing seminar on the project
Diagnosis, disease dynamics and intervention:
Salmonella Dublin and Paratuberculosis

CEPROS II-7

Table of Contents

Preface

The project "Diagnosis, disease dynamics and intervention: Salmonella Dublin and Paratuberculosis", with the acronym DubPar, was initiated in 2003 as a project in Centre for the Management of Animal Production and Health (CEPROS). The objective of the DubPar project as stated in the application can be found below. The DubPar seminar summarises the results obtained in the project, including the non-peer-reviewed eight papers presented in these proceedings. Also included is a list of publications resulting from the project or associated projects at the end of the proceedings.
Another important part of the DubPar project was to host the 8th International Colloquium on Paratuberculosis in Copenhagen in 2005. The 8ICP was a tremendous success with 360 participants from 34 countries and achieved a profit for the benefit of The International Association for Paratuberculosis.

Gregers Jungersen, DubPar Project Manager

Søren Saxmose Nielsen, Proceedings Editor

Objectives of the DubPar project

Diagnosis, disease dynamics and intervention: *Salmonella* Dublin and paratuberculosis

"DubPar"

The goal of this project is to deliver the tools, know-how and inspiration for the control and eradication of paratuberculosis and *Salmonella* Dublin from Danish cattle herds.

A national consensus on intervention strategies and recommendations among participating institutions and companies should be reached through a thorough discussion of results acquired from several veterinary and agricultural disciplines.

Completion of this goal will be reached by

- development of optimal tools for immunological and bacteriological diagnosis
- immunological and epidemiological research on dynamics of disease development in cattle with progressive paratuberculosis and carrier animals of *Salmonella* Dublin
- epidemiological research and modelling to identify contribution of host factors, environment and in-herd management factors on level of infection and effects of intervention
- intervention studies in selected herds
- generation of a "Manual for Advisors" for intervention against *Mycobacterium avium* subsp. *paratuberculosis* and *Salmonella* Dublin in field-trials

Participating institutions in the DubPar project

Most of the participating institutions have changed names during the project. Only the current names are given for each institution

The following institutions have participated in the project

The National Veterinary Institute (VET-DTU)
and
The National Food Institute (FOOD-DTU)
Technical University of Denmark
Bülowsvej 27
DK-1790 Copenhagen V

Department of Large Animal Sciences
Faculty of Life Sciences (KU-LIFE)
University of Copenhagen
Grønnegårdsvej 8
DK-1870 Frederiksberg C

Department of Animal Health, Welfare and Nutrition
Faculty of Agricultural Sciences (FAS-AU)
Aarhus University
P.O. Box 50
DK-8830

Danish Dairy Board
Danish Cattle Federation (DCF)
Frederiks Allé 22
DK-8000 Århus C

Project participants

Project manager
Senior Scientist, DVM, Ph.D. Gregers Jungersen, VET-DTU, e-mail: gju@vet.dtu.dk

Project participants
Dorte Lau Baggesen, Senior Scientist, DVM, Ph.D., FOOD-DTU, e-mail: dlb@vet.dtu.dk

Susanne Nedergård Grell, M.Sc. Ph.D., VET-DTU

Håkan Vigre, Senior Scientist, DVM, Ph.D., VET-DTU, e-mail: hvi@vet.dtu.dk

René Bødker, Epidemiogist, M.Sc., Ph.D., VET-DTU, e-mail: reb@vet.dtu.dk

Anne Braad Kudahl, Scientist, M.Sc., Ph.D., FAS-AU, e-mail: AnneB.Kudahl@agrsci.dk

Liza Rosenbaum Nielsen, Associate Professor, DVM, Ph.D., DipECVPH, KU-LIFE, e-mail: lrn@life.ku.dk

Annette Kjær Ersbøll, Associate Professor, M.Sc., Ph.D, KU-LIFE, e-mail: ake@life.ku.dk

Søren Saxmose Nielsen, Associate Professor, DVM, Ph.D., DipECVPH, KU-LIFE, e-mail: ssn@life.ku.dk

Erik Rattenborg, Epidemiologist, DVM, Ph.D., DCF, e-mail: era@mejeri.dk

Introduction

Hans Houe

University of Copenhagen, Faculty of Life Sciences (LIFE), Dept. of Large Animal Sciences

THE CEPROS CONCEPT

The project presented in this proceeding has been part of the Research Centre for the Management of Animal Production and Health (CEPROS). The research centre was founded on September 1st, 1996 as a result of an agreement between four institutions: the Danish Institute of Agricultural Sciences (now Faculty of Animal Sciences, University of Århus), the Danish Veterinary Laboratory (now National Veterinary Institute and National Food Institute, Technical University of Denmark), the Danish Veterinary Institute for Virus Research (now National Veterinary Institute, Technical University of Denmark) and the Royal Veterinary and Agricultural University (now Faculty of Life Sciences, University of Copenhagen). The establishment of the centre was a result of recommendations in the report concerning a proposal for a national strategy for public sector research and development in agriculture (report number 1274, June 1994). CEPROS was initially founded for a 5-year period, but later extended for another 5 years until the end of 2006. In the extended period, also Institute for Food and Resource Economics, now at University of Copenhagen participated.

The purpose of the centre was to coordinate fundamental research with applied research and to integrate veterinary and production-oriented animal research, in such a way as to promote knowledge to how profitable production of livestock can best be carried out with consideration for the health and welfare of livestock, as well as reducing the usage of medication. The idea was to encourage horisontal integration between research disciplines as well as vertical integration between basic and applied research.

CEPROS was a “centre without walls” meaning that it was located as an integrated part of the participating institutions, and the departments, which participated in the centre's research organisation.

One of the basic ideas behind integrated research is that the integration will provide research that one research discipline or a single researcher cannot perform alone. Thus, there will be synergy of creating new ideas and methodology and also to put existing methodology into a new context.

EXPERIENCES IN CEPROS

During the first years of managing CEPROS projects, a number of experiences were obtained in relation to the concept of integration, importance of attitude of the researchers and the project organisation and management. Among the main recommendations established from these experiences are not to integrate too many research disciplines at the same time. If the individual researcher has to cope with concepts and methodology from many disciplines at the same time it can be overwhelming and demotivating. In order to create well-functioning integrated research groups, it is necessary that the project participants have some knowledge of other participating disciplines i.e. can look into the other disciplines without loosing the depth and expertise of their own discipline. In doing so, they will gain respect and understand the necessity of other disciplines for the whole project and feel responsible for the whole project. Some degree of concept clarification is usually necessary. In such an environment new ideas and new ways of presenting the problems can come forward. A well functioning organisation is necessary to maintain the will and power of interdisciplinary research projects.

CEPROS AND THE “DUBPAR” PROJECT

The current project “Diagnosis, disease dynamics and intervention: *Salmonella* Dublin and paratuberculosis (“DUBPAR”)” was founded in the second period of CEPROS. When looking at all the part projects individually and how they have been linked together over time, there is a lot of evidence that the CEPROS concept has been very much in focus in this particular project. Thus, there has been horizontal integration between a balanced number of research disciplines including bacteriology, immunology, epidemiology, economics and disease control. There has been a clear line of vertical integration from establishment of diagnostic tests, modelling disease dynamics, identification of risk factors, economic evaluation up until establishment of intervention studies and control programs. The programmes are accompanied by a detailed manual for the advisors and farm owners. It is very obvious that the integrative approach has created a lot of synergy between the participating researchers and the collaboration with international researchers has further added to this process. I encourage that the proceeding is read from the perspective of integration and see how the individual parts fit into the whole context.

Evaluation of *Salmonella* Dublin bacteriological culture method

Dorte Lau Baggesen[1], Liza Rosenbaum Nielsen[2], Gitte Sørensen[1], Rikke Berreda[1], Rene Bødker[3], Jeffrey Hoorfar[1] and Annette Kjær Ersbøll[2]

[1]Technical University of Denmark, National Food Institute; [2]University of Copenhagen, Faculty of Life Sciences; [3]Technical University of Denmark, National Veterinary Institute

ABSTRACT

Difficulty in isolation of *Salmonella* (*S.*) Dublin promotes research in relation to improve sensitivity of bacteriological methods. Evaluation of different conventional strategies for isolation of *S.* Dublin from cattle herds showed the performance of strategies differed significantly among different bacterial strains and sources of faecal material. Detailed investigation of the faecal flora (pathogens and normal flora) and the interaction with chemical factors may result in developing an improved method for detection of *S.* Dublin. Overall, the Modified Semi-solid Rappaport Vassiliadis (MSRV)-culture medium had the most reliable detection capability, whereas detection with Selenite Cysteine-broth and Müller-Kauffmann Tetrathionat broth with Novobiocin in combination with both indicative medias varied more and rarely reached the same level of detection as MSRV in this experiment. Xylose Lysine Deoxycholat agar was the most reliable indicative media compared to Brilliant Green Agar, especially in combination with MSRV.

Use of an automatic method based on immunomagnetic separation (PATHATRIX^{TM}) identified the same positive samples as the conventional standard method and as the automatic methods were both more expensive and time consuming the new method would not be a cost-effective alternative to replace the current ISO standard method for *S.* Dublin detection.

Detection of *S.* Dublin by real-time PCR identifying the presence of a *Salmonella* specific gene, the *ttr*-gene encoding the tetrathionate respiration showed improved sensitivity compared to the standard culturing method. In a study including 10 animals, four samples representing three animals were positive by traditional bacteriological culturing, whereas 18 samples representing 4 animals where positive by PCR including those that were positive by traditional culture. In another study, the culturing method found 3 herds and 9 manure tanks positive whereas the PCR method found 7 herds and 15 manure tanks positive in 9 confirmed *S.* Dublin outbreak herds. Diagnosis by real time PCR opens, however, for the discussion on the applicability and reliability of diagnostic methods where the positive results are not verified by isolation of the causable organism. Therefore, further investigation will be performed to optimise the PCR-test and evaluate its specificity and usefulness in relation to diagnosis and control of *S.* Dublin in the cattle production.

INTRODUCTION

Diagnosis of *Salmonella* (*S.*) Dublin infection in cattle is very challenging. Many studies report difficulties in isolation of the bacteria even when material from individuals or herds that are known to be infected is investigated (Richardson and Fawcett, 1973; Hinton, 1974; House et al., 1993). Low isolation frequency is also reported when materials from individuals or herds that are identified as “probably infected” by serological analysis is investigated (House et al., 1993; Hoorfar et al., 1996; Veling et al., 2000; Veling et al., 2002; Nielsen et al., 2004; Nielsen and Ersbøll, 2005). Diagnosis of the infection is, however, important in relation to both surveillance and control.

In Denmark, a surveillance programme based on serological investigation of bulk tank milk samples (dairy herds) and blood samples collected primarily at slaughter (non-dairy herds) by a serogroup D LPS ELISA (Hoorfar et al., 1995; Nielsen et al., 2003; Nielsen and Ersbøll, 2005; Nielsen et al., 2006), has been in place since 2002. Based on the serological results herds are assigned to one of two levels as “probably not *S.* Dublin infected” (Level 1) or “probably *S.* Dublin infected” (Level 2).

In herds assigned to Level 2 there is a need for further diagnostic analysis in order to establish intervention procedures to control the infection in the herd and to convince the producer of the presence and the significance of the infection. Serological analysis is applied for evaluation of the presence and spread of the infection among different groups of individuals within the herd and for identification of suspected carrier animal, but the difficulties in isolation of the *S.* Dublin bacteria from the herd/individuals frustrate producers and advisers as this would be the final prove of infection. Also, food safety authorities have a great interest in more sensitive bacteriological methods be able to correctly identify high risk herds.

The difficulties in isolation the bacteria from cattle material e.g. faeces may have several explanations. The main reason is that the excretion of bacteria to faeces in general is low and intermittent or non-existing in individuals that do not suffer from salmonellosis or have been clinically affected in the previous weeks before testing. It is therefore important to realize that detection of infected animals is another task than identification of excreting animals and that the same method not necessarily can be used for both aims. In addition, no bacteriological isolation method is 100% sensitive saying that even though one or few bacteria are present in the sample, these will not in all cases be enriched to level over the detection limit during the culturing procedure. The inadequate enrichment can be caused by competition with the natural flora of the

material or presence of inhibitory substances. Further, it has been shown that the bacteria may be clustered in the sample which also lowers the probability of recovering the bacteria when sampling small amounts (Cannon and Nicholls, 2002).

In the project "Diagnosis, disease dynamics and intervention: *Salmonella* Dublin and paratuberculosis" we have focused on methods to improve the analytical sensitivity of the bacteriological method performed in relation to investigation in cattle herds. The aim has been to improve the methods for detection of animals or groups of animals that constitute a high risk of excreting the bacteria in faeces and thereby improve the tools for analysing spread of infection among different groups of animals and the risk of introduction the bacterial contamination to the slaughterhouse. The activities have included i) evaluation of different conventional isolation strategies, ii) evaluation of an automated method for isolation of *Salmonella* based on immunomagnetic separation (IMS) and iii) detection of *Salmonella* by real-time PCR.

DESIGN AND RESULTS OF RESEARCH ACTIVITIES

Evaluation of different conventional isolation strategies

The aim of this part was to analyse the relative importance of different biological and technical factors that influenced the analytical sensitivity of conventional culture methods for detection of *S.* Dublin in cattle faeces (Baggesen et al., 2007).

In an experimental set up, faeces samples collected from six adult bovines from different *Salmonella* negative herds were split into sub-pools and spiked with three strains of *S.* Dublin at a concentration level of approximately 10 CFU/g faeces. Each of the 18 strain-pools was divided into two sets of triplicates of four volumes of faecal matter (1, 5, 10 and 25 g). The two sets were pre-enriched in buffered peptone water with and without Novobiocin, followed by combinations of culture media (Modified Semi-solid Rappaport Vassiliadis (MSRV), Selinite Cystein broth (SC) and Müller-Kauffmann Tetrathionat broth with Novobiocin (MKTTn)) and selective media (Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholat agar (XLD)). The analytical sensitivity of each combination and sources of variation in detection were determined by a split-plot statistical model accounting for correlation between samples on pool and strain level.

Through the study, it was concluded that biological factors such as faecal origin and *S.* Dublin strain influenced the analytical sensitivity more than technical factors such as selective or indicative media used or the volume of the faecal sample tested. Overall, the MSRV-culture medium had the most reliable detection capability, whereas detection with Selenite Cysteine-broth and MKTTn varied more and rarely reached the same level of detection as MSRV in this experiment. XLD was the most reliable indicative media compared to BGA, especially in combination with MSRV. The present study therefore support the choice of MSRV in combination with XLD and an additional indicative medium as gold standard for detection of *Salmonella* from the primary animal production (ISO 7265/2006 Annex D).

The study showed that for MSRV-culture medium and Xylose Lysine Decarboxylase agar as the indicative medium, the sensitivity of the faecal culture method may be improved by focusing on the strain variations and the ecology of the faecal sample. Detailed investigation of the faecal flora (pathogens and normal flora) and the interaction with chemical factors may result in developing an improved method for detection of *S.* Dublin.

Evaluation of an automated method for isolation of Salmonella based on immunomagnetic separation (IMS)

A novel method called PATHATRIX™ which is based on immunomagnetic separation technique has been developed to detect *Salmonella* in different matrices (Matrix MicroScience Ltd, UK). This new method is, in theory, able to detect the bacteria from the entire pre-enriched sample (225 ml) instead of the 100 µl that is analysed in the traditional MSRV-culturing method. This means that the IMS method could have a higher sensitivity because of this up-concentration. The PATHATRIX™ system re-circulates the entire pre-enriched sample by aid of peristaltic pumping technology. The sample is passing by magnetic beads coated with polyclonal antibody against *Salmonella*. After running the sample through the PATHATRIX™ system, the beads are plated onto agar plates to identify and serotype the *Salmonella* caught with the beads.

The aim of this part of the activities was to compare the sensitivity and the applicability in relation to workload and cost of the PATHATRIX ™ system with the MSRV based ISO standard method for bacterial culture method for the detection of *S*. Dublin (ISO 7265/2006 Annex D). The samples applied for the comparison originated from ten animals selected as suspected carrier animals based on high antibody levels to *S.* Dublin LPS in ELISA (Lomborg and Nielsen, 2006). A total of 281 faecal, 111 milk, 6 foetus and 154 organ samples from ten cattle were analysed. The results obtained by both methods were identical. *Salmonella* was isolated from four samples representing three animals (mammary lymph node, spleen and liver (2)). Only organ samples were found positive in these two methods. *Salmonella* was not isolated from any other samples using IMS and traditional bacteriological culturing.

The traditional culturing method is able to detect *Salmonella* in four days and the IMS method is able to detect the bacteria in only three days. The PATHATRIX™ is, however, an expensive method with a cost of approximately 9 Euro per test for materials not including culturing media and labour expenses.

PATHATRIX™ needs a lot of labour time to get the samples and tubes installed correctly for every sample making the traditional culturing method more easy to carry out at the laboratory.

Although the material used for comparison was rather weak due to the low number of positive samples, the results did not indicate that the PATHATRIX™ would be a cost-effective alternative to replace the current ISO standard method for *S*. Dublin detection.

Detection of Salmonella by real-time PCR

In the last part of the activities, the aim was to perform an initial evaluation of the performance of a real-time *Salmonella* PCR assay (Malorny, et al, 2004; Reynisson et al, 2005) that has been set up in the laboratory for analysis of pork and poultry meat. In this real-time PCR, the presence of a *Salmonella* specific gene, the *ttr*-gene encoding the tetrathionate respiration is detected (Malorny et al, 2004). The evaluation was based on comparison with the MRSV based ISO standard method for bacterial culture method for the detection of *S.* Dublin (ISO 7265/2006 Annex D).

In the real time-PCR test, DNA was extracted from 1 ml pre-enrichment buffer by use a KingFisher magnetic beads automatic DNA isolation system. Primers and sequences were described in Malorny et al.,(2004) and running conditions as described by Berreda (2006). The tests were performed in an Mx3005p instrument (Stratagene, La Jolla, USA) and the Mx3005p v2.02 Build 268 software was used for data analysis including establishment of a threshold (Ct)-value for the interpretation of a positive result. All samples were analysed in triplicates. Samples showing fluorescence signal Ct $\leq$ 36 in two out of three triplicates were regarded positive. Samples giving results >36 or no Ct-value were regarded negative. Samples where one or two replicates had a Ct-value >36 were run in the PCR assay again.

Three different materials were used for evaluation of the real time-PCR method:

1. Material from high titre cows: 368 samples (164 faecal samples, 49 milk samples, 154 organ samples, 1 foetus) from ten animals selected as suspected carrier animals based on high antibody levels to *S.* Dublin LPS in ELISA. (Lomborg, 2006)
2. Material from herds with clinical outbreaks of salmonellosis: 159 manure samples originating from 9 herds with recent *S.* Dublin-confirmed clinical infection (18 different manure tanks were sampled in triplicates three times with approximately 5 weeks in between the sampling rounds).
3. Material from herds considered free of *Salmonella* infection: 115 faecal samples from 6 herds selected as not *S.* Dublin infected based on repeated serological monitoring (quarterly individual milk sampling of all lactating cows and biannual sampling of all young stock in the herd for three years prior to collection of the faecal samples with no seropositive reactions measured by ELISA).

Table 1. *Salmonella* analyses of samples from cattle herds by conventional culture and real-time PCR

		Real time PCR positive	Real time PCR negative	Relative sensitivity	
				Culture	PCR
Material from high titre cows (no. of samples)	Culture positive	4	0	4/18 = 22%	4/4 = 100%
	Culture negative	14	350		
	Sum	18	350		
Material from herds with clinical outbreak of salmonellosis (no. of samples)	Culture positive	46	6	46/87 = 53%	46/52 = 88%
	Culture negative	41	66		
	Sum	87	72		
				Specificity	
				Culture	PCR
Material from herds free of *Salmonella* infection (no. of samples)	Culture positive	0	0	115/115 = 100%	115/115 = 100%
	Culture negative	0	115		
	Sum	0	115		

Results of conventional culture and real-time PCR analyses of the different materials are summarised in Table 1. The two materials first mentioned were selected as presumable *Salmonella* positive and *S.* Dublin was isolated by culturing from both materials even though the prevalence of positive samples was low. For both materials more samples were positive by PCR analysis. In the first material, four samples representing three animals were positive by culturing where as 18 samples representing four animals where positive by PCR. In the second material, the culturing method found three herds and nine manure tanks positive where

as the PCR method found seven herds and 15 tanks positive. All triplicates were not positive in all cases where a tank was found positive, but a tank was assigned positive if two out of three triplicates were positive. Culture analysis of the second material was semi-quantitative and an association with higher Ct-values at lower *Salmonella* levels was demonstrated.

The third material was selected as originating from presumable *Salmonella* negative cattle herds, which was confirmed by only negative results of analysis of 115 samples by both conventional culture and real time-PCR. This demonstrated a high specificity of both methods.

DISCUSSION, CONCLUSION AND PERSPECTIVES

The presented activities have repeatedly demonstrated the difficulty in isolation of *S.* Dublin even from material from herds known to be *S* Dublin infected and herds with clinical outbreaks of *S*. Dublin. Looking into conventional culture procedure, the method which recently has been appointed as official standard method for detection of *Salmonella* from primary animal production, showed the highest sensitivity in these studies. The performance of different culturing strategies varied markedly between different strains of *S.* Dublin and sources of faeces indicating that biological factors influenced the analytical sensitivity more than technical factors.

Alternative methods have been suggested for improving the sensitivity of detection of *S.* Dublin from cattle. An improved automated method based on immunomagnetic separation (PATHATRIX^{TM}) was investigated with discouraging result. In contrast, the initial evaluation of real time PCR indicates an increased sensitivity with identification of more positive samples and more positive animals. Diagnosis by real-time PCR opens for the discussion on the applicability and reliability of diagnostic methods where the positive results are not verified by isolation of the causable organism. Initial specificity investigation was promising making real-time PCR interesting in areas where ELISA lacks validity due to impaired specificity. However, we also found that the real-time PCR is not always positive when it was possible to detect bacteria by conventional culture. Therefore, further investigation will be performed in order to optimise the real-time PCR test and evaluate its usefulness in relation to diagnose and control of *S.* Dublin in the cattle production.

REFERENCES

Baggesen, D.L., Nielsen, L.R., Sørensen, G., Bodker, R. and Ersbøll, A.K., 2007. Growth inhibitory factors in bovine faeces impairs detection of *Salmonella* Dublin by conventional culture procedure. J Appl. Microbiol. (Available online: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2672.2007.03292.x)

Berrada, R.P., 2006. Improved detection of *Salmonella* Dublin in samples from cattle. Thesis: Master of Science, University of Copenhagen and Danish Institute for Food and Veterinary Research.

Cannon, R.M., Nicholls, T.J., 2002. Relationship between sample weight, homogeneity, and sensitivity of fecal culture for *Salmonella enterica*. J. Vet. Diagn. Invest. 14, 60-62.

Hinton, M., 1974. Isolation of *Salmonella* Dublin from Fecal Swabs from Cattle. Br. Vet. J. 130, R31-R32.

Hoorfar, J., Lind, P. and Bitsch, V., 1995. Evaluation of an O antigen enzyme-linked immunosorbent assay for screening of milk samples for *Salmonella* dublin infection in dairy herds. Can. J. Vet. Res. 59, 142-148.

Hoorfar, J., Wedderkopp, A. and Lind, P., 1996. Comparison between persisting anti-lipopolysaccharide antibodies and culture at postmortem in salmonella-infected cattle herds. Vet. Microbiol. 50, 81-94.

House, J.K., Smith, B.P., Dilling, G.W. and Roden, L.D., 1993. Enzyme-linked immunosorbent assay for serologic detection of *Salmonella* dublin carriers on a large dairy. Am. J. Vet. Res. 54, 1391-1399.

Lomborg, S.R., Nielsen, L.R., 2006. Immune suppression of cattle suspected as carriers of *Salmonella* Dublin. *In*: Proceedings of the International Symposium Salmonella and Salmonellosis, Ploufragan, France, May 2006.

Malorny, B., Paccassoni, E., Fach, P., Bunge, C., Martin, A., Helmuth, R., 2004. Diagnostic Real-Time PCR for Detection of Salmonella in Food. Appl. Environ. Microbial., 70, 7046-7052.

Nielsen, L.R., Ersbøll, A.K., 2005. Factors associated with variation in bulk-tank-milk *Salmonella* Dublin ELISA ODC% in dairy herds. Prev. Vet. Med. 68, 165-179.

Nielsen, L.R., Rattenborg, E., Nielsen, J., 2003. National surveillance program for *Salmonella* Dublin in Danish cattle. In: Proceedings of the 10th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE), Viña del Mar, Chile, November 2003, no. 847.

Nielsen, L.R., Rattenborg, E., Nielsen, J., 2006. Development of the National Surveillance Programme for *Salmonella* Dublin in Danish cattle. In: Proceedings of the 11th International Symposium for Veterinary Epidemiology and Economics (ISVEE), Cairns, Australia, August 2006, no. 870.

Nielsen, L.R., Toft, N. and Ersboll, A.K., 2004. Evaluation of an indirect serum ELISA and a bacteriological faecal culture test for diagnosis of *Salmonella* serotype Dublin in cattle using latent class models. J Appl. Microbiol., 96, 311-319.

Reynisson, E., Josefsen, M.H., Krause, M., Hoorfar, J., (2005) Evaluation of probe chemistries and platforms to improve the detection limit of real-time PCR. J Microbiol Methods., 66, 206-16.

Richardson, A., Fawcett, A.R., 1973. *Salmonella*-Dublin Infection in Calves - Value of Rectal Swabs in Diagnosis and Epidemiological Studies. Br. Vet. J. 129, 151-156.

Veling, J., Barkema, H.W., van der Schans, J., van Zijderveld, F. and Verhoeff, J., 2002. Herd-level diagnosis for *Salmonella enterica* subsp. *enterica* Serovar Dublin infection in bovine dairy herds. Prev. Vet. Med. 53, 31-42.

Veling, J., van Zijderveld, F.G., Zijderveld-van Bemmel, A.M., Barkema, H.W. and Schukken, Y.H., 2000. Evaluation of three newly developed enzyme-linked immunosorbent assays and two agglutination tests for detecting *Salmonella enterica* subsp. *enterica* Serovar Dublin infections in dairy cattle. J. Clin. Microbiol., 38, 4402-4407.

Challenging the traditional methods for detection of *Salmonella* Dublin carrier animals

Liza Rosenbaum Nielsen[1], Dorte Lau Baggesen[2] and Annette Kjær Ersbøll[1].

[1]*University of Copenhagen, Faculty of Life Sciences (LIFE), Dept. of Large Animal Sciences;*
[2]*The Technical University of Denmark, National Food Institute (FOOD-DTU)*

ABSTRACT

Control or eradication of *Salmonella (S.)* Dublin from cattle herds requires intervention by closing transmission routes and minimizing the exposure to the bacteria in general.Traditionally, test-and-cull strategies to remove persistently infected cattle have been considered important elements of interventions. However, these recommendations have mostly been based on studies performed under experimental conditions which do not correspond to the real life situation in infected herds. They may overemphasize the importance of culling persistently infected animals or the simplicity of detecting true carriers. In this project, three studies were carried out to challenge the hypothesis that carrier animals can be detected with reasonable accuracy using repeated antibody measurements on serum or milk samples.

In the first and largest study, we found a significantly higher probability of *S.* Dublin positive faecal samples from animals with both recent and long-term high antibody measurements than from animals with low antibody measurements in either all or the most recent sample. Overall, the probability of culture positive faecal samples was between 0.1-10% at any given point in time in chronically infected herds. Young age was strongly associated with highest risk of shedding. The probability estimates should be adjusted due to poor sensitivity of the faecal culture method due to intermittent shedding. Thus, the true probability of bacterial shedding at any given point in time is more likely to be 2-5 times higher than estimated in this study.

In the second study, we found very low frequency and concentrations of *S.* Dublin bacteria in faecal material from adult cows in two chronically infected dairy herds in spite of intensive over the Fall where shedding is supposed to be highest. We found no associations between the proportion of shedding animals and serological profile groups, parity, stage of lactation or faecal scores.

In the third study, no shedding of *S.*.Dublin was found in faeces or milk during from 9 adult cattle suspected of being persistently infected carriers based on persistently high antibody measurement over at least 180 days. *S.* Dublin bacteria were recovered from tissue samples from three animals (30%) in the mammary lymph nodes, spleen and liver.

Overall, the studies indicate that the risk of finding high shedding animals in chronically infected dairy herds is low. Young stock with high antibody measurement appear to pose the highest risk of shedding, whereas older cows are not good candidates for culling based on serology or combined serology and faecal culture.

INTRODUCTION

Controlling *S.* Dublin in an infected herd requires intervention through identification and closure of open transmission routes. The transmission routes may vary from farm to farm depending on the structure of the barns, herd size, age groups and group sizes, contact between age groups and the hygiene of the barn environment. It is generally accepted that calves are most frequently affected and symptoms are usually seen below the age of six months (Richardson, 1974). This is an important age group to focus on when attempting to eradicate or control the infection within a herd. Since the early 1970'ies, it has also been a general practice to advice test-and-cull of persistently infected animals, because these are assumed to pose a high risk of continuous or intermittent re-infection of the herd, thereby lowering the probability of successful intervention. From the early 1990'ies, the detection was advised to be based on serology using repeated sampling of individual cattle (Richardson, 1973; Sojka et al., 1974; Smith et al., 1989; Spier et al., 1991; Smith et al., 1992; Nielsen and Vestergaard, 1992; House et al., 1993; Jensen et al., 1994; House et al., 2001; Jensen et al., 2004).

Several methods have been suggested to detect actively or latently infected carriers either by repeated antibody measurements (serological profiles) in serum or milk samples, by a combination of antibody measurements and repeated bacteriological culture or by repeated bacteriological culture alone (Richardson, 1973; Spier et al., 1990; Smith et al., 1992; Veling, 2004). Further, it has been suggested that immunosuppression of persistently infected carrier animals leads to reactivation of the infection followed by increased shedding of bacteria (Gronstol et al., 1974; Spier et al., 1991).

Carrier detection is expensive and work intensive. Using the traditional recommendations, about culling animals with high antibody measurements in two or three samples over 90-120 days, results in a long list of cows for culling in long-term infected herds. Often, it is not possible for the farmer to cull that many animals if he wants to keep the production level. Further, there are indications from previous studies that not all of the suspected animals actually pose a risk in the herd (Hoorfar et al., 1996). Thus, there is a need to better understand the importance of the carrier animals in relation to transmission of the infection and the

limitations of the traditional methods of detecting carriers. In the Dub-Par-project, we have tried to challenge the methods of detection of carrier animals by using repeated *S.* Dublin antibody measurements and bacteriological culture in three studies with different study designs:

Study 1 was an observational study based on repeated cross-sectional studies in dairy herds that were selected for the study based on high bulk-tank milk antibodies. The bulk-tank milk measurements suggested that the infection was present in the herd from the beginning of the study and this was confirmed with bacteriological culture. All herds were visited five times with three months intervals. At each visit all animals in the barns had a rectally collected faecal sample and either a blood or an individual milk sample collected depending on age and lactation status. The data was used to estimate the probability of finding *S.* Dublin culture positive faecal samples from animals with different types of serological profiles in all animals above seven months of age which is approximately the time it would be possible to start grouping animals into risk groups based on repeated antibody measurements.

Because it could be argued that the sensitivity of the bacteriological method was too poor and the frequency of sampling was too small in Study 1 to detect those carrier animals that were shedding intermittently, Study 2 was carried out as an observational cohorte study in which two dairy herds that were known to have been infected with *S.* Dublin for at least one year were visited four times with four weeks intervals. For four consecutive days at each of the four visits all of 58 cows had faecal samples collected twice per day for bacteriological culture. They also had milk samples collected monthly during the same period for determination of their repeated antibody status. The data was used to compare the proportion of shedding animals between different types of serological profiles in cows.

Because it could be argued that the two dairy herds that were selected for Study 2 had too high environmental contamination and not enough control over the infection to properly study truly persistently infected animals, Study 3 was carried out as an experimental study in which 9 adult cattle with continuously high antibody measurements for a minimum of 180 days were purchased from dairy herds that participated in an intervention trial. The animals were brought to an isolation facility where they were immunosuppressed with high doses of dexamethasone sodium phosphate. Faecal and milk samples were collected 2-4 times per day and cultured for *S.* bacteria. After one-two weeks in isolation the animals were euthanized and at autopsy samples from 15 organs were collected for bacteriological culture.

MATERIALS AND METHODS

Bacteriological culture method

Faecal samples were examined for presence of *Salmonella* bacteria using 25 g of faecal material that was mixed in 225 ml peptone buffer and left for pre-enrichment at 37ºC for 18-24 hours. Inoculation of 0.1 ml test material onto Modified Semi-solid Rappaport Vassiliadis Medium Base (MSRV-agar) plates and 1 ml test material into 9 ml of selenite-cystine was followed by incubation for 18-24 hours at 41.5ºC. Material from the selenite-cystine tubes was inoculated on modified Brilliant-green Phenol-red Lactose Sucrose agar (BPLS-agar) plates and incubated at 37ºC for 18-24 hours. Positive test results from MSRV were inoculated onto BPLS-agar plates and confirmed using Triple Sugar Iron agar-tests and Lysine-Iron-agar tests. Serotyping and confirmation of positive isolates were conducted at the National Food Institute. For Study 1, pooled faecal samples were used as described later.

Antibody measurements by ELISA

The serum *Salmonella serogroup D*-ELISA used in this study was performed as a previously described ELISA method (Hoorfar et al., 1994; Nielsen and Ersbøll, 2004). The ELISA detects antibodies directed against *S.* Dublin O-antigen based lipopolysaccharide. An ODC%-value, which is a background corrected proportion of the test sample OD to a positive reference sample, was calculated as follows:

$$\text{ODC\%} \quad \frac{\left(\overline{OD}_{\text{sample}} - \overline{OD}_{\text{neg ref}}\right)}{\left(\overline{OD}_{\text{pos ref}} - \overline{OD}_{\text{neg ref}}\right)} * 100$$

where $\overline{OD}_{\text{sample}}$ is the mean value of two test wells, $\overline{OD}_{\text{neg ref}}$ and $\overline{OD}_{\text{pos ref}}$ are the mean values of four reference wells in the ELISA plates. In theory, ODC% values can go below 0, but these are rounded off to 0. The scale of ODC% used in practice runs from 0 to approximately 160 ODC%.

Study 1: Probability of finding Salmonella Dublin bacteria in faecal samples in four risk groups classified by repeated antibody measurements in cattle from 14 persistently infected dairy herds.

Data was used from 14 dairy herds that participated in a large research project known as the Kongeåproject in 2000-2002 (Andersen et al., 2000; Nielsen, 2003). These herds were selected for the study based on bulk-tank milk antibody measurements above 50 ODC% early in year 2000. They were later confirmed to be infected with *S.* Dublin by at least one bacteriologically positive faecal culture. All herds

except one were visited five times with approximately three months between each visit in the period March 2000 to January 2002. The last herd was only visited four times due to practical problems with the last planned herd visit. At each visit all animals that were not kept outside for grazing were sampled. The samples consisted of rectally collected faecal samples, blood samples from young stock and dry cows and individual milk samples collected at milking from lactating cows. The samples were brought to Steins Laboratory in Ladelund less than two hours driving distance from the herds immediately after sampling was finished. At the laboratory faecal samples were pooled 5 at the time using 5 g per sample that was mixed to a 25 g pool that was then cultured as described above. If the pool was found positive of *Salmonella* the individual samples were cultured using 25 g of faecal material to try to locate those animals that were positive in the pool. It has been estimated that using the pooling procedure may lower the sensitivity of the culture method to approximately half of the sensitivity of the method using individual samples as the first step.

ELISA results from animals below the age of 84 days (12 weeks) were not used, because the sensitivity and specificity of the test are known to be compromised by impaired capability of antibody production in calves below the age of 11-12 weeks (Da Roden et al., 1992) and maternally derived antibodies from colostrum . Based on practical experience with the test results and literature, ELISA results from animals from 84 days were used to group every individual into risk groups based on their serological profiles in the last up to four consecutive samples before the study period ended using the following criteria*:

Risk group 1: At least two samples available.
The average of the last (up to) four samples and the most recent sample was above 80 ODC%.
At least 120 days between the first and the last sample above 80 ODC%.
This group was considered high risk of being a persistently infected carrier animal.

Risk group 2: The most recent sample above 50 ODC% or
the average of the last up to four samples above 50 ODC% (but not above 80 ODC%).
This group was considered to have moderate risk of being a persistently infected carrier animal.

Risk group 3: The average of the last up to four samples between 25-50 ODC% or
the most recent sample between 25-50 ODC%.
This group was considered very low risk of being a persistently infected carrier animal.

Risk group 4: The average of the last up to four samples <50 ODC% and
the most recent sample <25 ODC%.
This group was considered low risk of being infected.

* few animals did not fit into these criteria and had to be manually placed into the groups.

Using these definitions, an animal that was only sampled once could only be grouped in Risk Group 2, 3 or 4 depending on whether that one ELISA measurement was above or below 25 and 50 ODC%. The age was recorded at the last sample. Having approximately three months between each sample date, the definition of the risk groups would be based on up to one year's worth of samples from the animals. However, many animals did not have four samples collected, either because they entered or left the herd during the study period or because they were out on pasture during the summer period and could not be sampled. In those rare cases where the animal was sampled with both serum and milk samples on the same data, the serum sample was used in the definition of the profile groups. All animals above the age of seven months at the last sample event were included in the dataset for analysis. This was to avoid including animals that could not have been detected as carrier animals yet due to the 120 days between samples requirement.

Using logistic regression it was tested whether the probability of finding at least one bacteriological culture positive faecal sample on any of the sample dates where the animals were above seven months old was associated with the risk groups and the age of the animal at the last sample. The GENMOD procedure in SAS® version 9.1 was used for the statistical analyses.

Study 2: Frequent testing for Salmonella Dublin bacteria in faeces from cows with different serological profiles in two persistently infected dairy herds

Faecal samples from 58 dairy cows from two herds with chronic *S.* Dublin infections were collected over four sampling rounds with four weeks intervals. Each cow was sampled twice daily for four days in a row. The cows were selected to represent different serological profiles in milk samples collected during the year before the sampling rounds were initiated. Most cows were milk-sampled at least three times with three month intervals. All faecal samples were tested for presence of *Salmonella* bacteria as described above at

the National Food Institute and the concentration of bacteria in positive samples was estimated using a semiquantitative method based on dilutions.

The cows were classified into three groups based on the repeated antibody measurements in milk samples as follows:
Group 1: All ELISA measurements >58 ODC%
Group 2: All ELISA measurements between 25 and 58 ODC% (and a max. of two between 0-158 ODC%)
Group 3: All ELISA measurements < 25 ODC%

Recordings of parity, stage of lactation (days in milk) and faecal consistency scores were collected for all animals. Statistical associations were tested between the proportion of shedding animals and the explanatory factors group, parity, stage of lactation and faecal scores using univariate chi-square tests and logistic regression in SAS® version 9 (Christensen, 2005).

Study 3: Immunosuppression of suspected Salmonella Dublin carrier cattle.

Nine adult cattle with persistently high antibody measurements (above 80 ODC%) for at least 180 days prior to the study period were purchased from four dairy herds that were taking part in intervention trials in the field. The animals transported for four to six hours and housed separately in isolation tie stalls for a period of 8-14 days. Clinical examinations were carried out daily. Faecal samples were collected four times a day and milk samples from the lactating cows were obtained twice daily for bacteriological cultures. Also a complete blood count was performed once daily. On day 3, 4 and 5 the animals had dexamethasone sodium phosphate 0.08 mg/kg administered intramuscularly. The animals were euthanatized and 14-16 tissue samples for bacteriological culture were obtained at necropsy (Lomborg, 2006).

RESULTS

Study 1

There were 9178 sample events including faecal culture results from animals above the age of 12 weeks; 78 of these events had a positive faecal culture result. 46 were faecal culture positive after the age of seven months. There were 4861 milk ELISA results combined with negative and 17 milk ELISA results combined with positive culture results. There were 4439 serum ELISA results combined with negative and 64 combined with positive faecal culture results. 203 animals were sampled with serum, milk and faecal samples simultaneously. Table 1 shows the distribution of total number of cattle above seven months of age and faecal culture positive animals in the four risk groups and overall. Both age and Risk Groups were significantly associated with the probability of faecal culture positive results ($p<0.0001$). In Table 1, Risk Groups with different letter symbols indicate groups with statistically different probabilities of faecal culture positive samples. Fig. 1 illustrates how the model predicted probability of being faecal culture positive depends on the risk group and age.

Table 1. Number and proportion of animals and faecal culture positive (FC-pos) animals in each of four risk groups based on serological profiles of all animals above the age of seven months across 14 dairy herds infected with *Salmonella* Dublin.

Risk groups	N in risk group (% of total)	FC-pos (% of N)
R1: Persistently high serology (>80 ODC%)[a]	206 (7.7%)	7 (3.4%)
R2: Moderately high serology (50-80 ODC%)[a]	704 (26.4%)	26 (3.7%)
R3: Medium to low serology (25-50 ODC%)[b]	574 (21.5%)	6 (1.0%)
R4: Low serology (25 ODC%)[b]	1185 (44.4%)	7 (0.6%)
Total	2669 (100%)	46 (1.7%)

[a,b] Risk Groups with different probabilities of having *S.* Dublin faecal culture positive samples

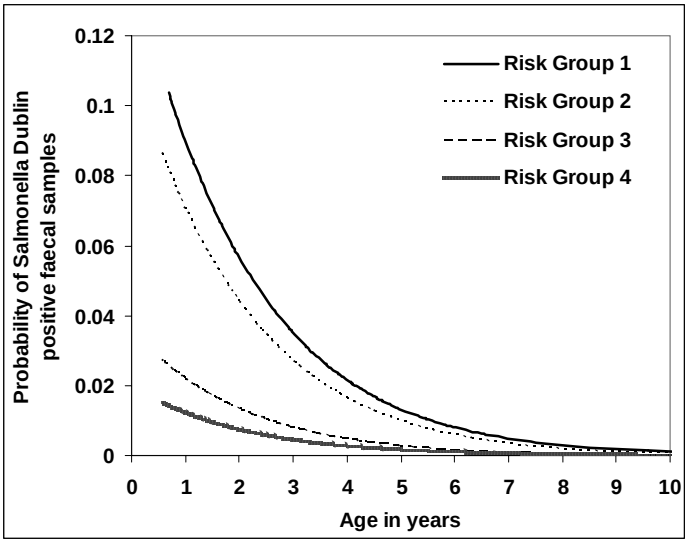


Fig 1. Model predicted probability of finding at least one *S.* Dublin positive faecal culture when sampling every three months over a period of one year vs. age of the animals in four different risk groups based on serological profiles. Risk Group 1 has the highest and most persistently high level of antibodies whereas Risk Group 4 has the lowest level of antibodies in the samples.

Study 2

A total of 1520 faecal samples were collected from the 58 dairy cows in this study. In total, 18 (1.2%) of these were found to contain *S.* Dublin bacteria. These positive samples came from 13 cows out of which only two cows were found to shed bacteria more than once. One cow shed bacteria three times, each time with 10 CFU/g faeces. There were 8 positive samples out of 557 in Group 1, 9 positive out of 735 in Group 2 and 1 positive out of 228 in Group 3. No association between the Groups and proportion of positive samples was found in this study.

The semiquantitative determinations found between 0-1000 CFU/g faeces with only one of the samples containing more than 100 CFU/g. There were no associations between proportion of shedding animals and parity, stage of lactation or faecal scores .

Study 3

Immune suppression was documented by the haematological profiles in all animals. No clinical signs or necropsy findings indicating salmonellosis were observed in any of the animals. No shedding of *S.* Dublin was found in faeces or milk during the period of this study. *S.* Dublin bacteria were recovered from tissue samples from three animals (30%) in the mammary lymph nodes, spleen and liver (Lomborg and Nielsen, 2006).

DISCUSSION

Whereas Study 1 showed that Risk Groups 1 and 2 consisting of animals with moderately high to persistently high antibody measurements for up to one year had significantly high probability of shedding *S.* Dublin bacteria the two risk groups with low antibody measurements, Study 2 was not able to support this association. It is likely that this discrepancy is because Study 2 looked at cows and the highest probabilities of shedding were found in young animals.

CONCLUSION

Comparing the results form the three studies suggest that in general the prevalence of shedding animals above the age of seven months – and in particular adult cows- in chronically infected herds is very low (0.1-2%) and that this is probably even lower in herds that undergo intervention. Thus, culling of carriers is most likely not important in comparison to hygiene of the environment and closure of transmission routes between young calves.

REFERENCES

Andersen, H.J., Aagaard, K., Skjøth, F., Rattenborg, E. and Enevoldsen, C., 2000. Integration of research, development, health promotion, and milk quality assurance in the Danish Dairy Industry. In: Salman,

M.D., Morley, P.S., Ruch-Galie, R. (Eds.), Proceedings of the 9th Symposium of the International Society of Veterinary Epidemiology and Economics., pp. 258-260.
Christensen, R.B., 2005. Udskillelsesdynamik af *Salmonella* Dublin hos kvæg fra kronisk inficerede besætninger og hos kalve fra udbrudsbesætninger. Veterinary Master Thesis. The Royal Veterinary and Agricultural University.
Da Roden, L., Smith, B.P., Spier, S.J. and Dilling, G.W., 1992. Effect of calf age and Salmonella bacterin type on ability to produce immunoglobulins directed against Salmonella whole cells or lipopolysaccharide. Am. J. Vet. Res. 53, 1895-1899.
Gronstol, H., Osborne, A.D. and Pethiyagoda, S., 1974. Experimental Salmonella infection in calves. 1. The effect of stress factors on the carrier state. J. Hyg. 72, 155-162.
Hoorfar, J., Feld, N.C., Schirmer, A.L., Bitsch, V. and Lind, P., 1994. Serodiagnosis of *Salmonella* dublin infection in Danish dairy herds using O-antigen based enzyme-linked immunosorbent assay. (Published erratum appears in Can. J. Vet. Res. 1995, 59 p. 25). Can. J. Vet. Res. 58, 268-274.
Hoorfar, J., Wedderkopp, A. and Lind, P., 1996. Comparison between persisting anti-lipopolysaccharide antibodies and culture at postmortem in salmonella-infected cattle herds. Vet. Microbiol. 50, 81-94.
House, J.K., Smith, B.P., Dilling, G.W. and Roden, L.D., 1993. Enzyme-linked immunosorbent assay for serologic detection of *Salmonella* dublin carriers on a large dairy. Am. J. Vet. Res. 54, 1391-1399.
House, J.K., Smith, B.P., O'Connell, K. and VanMetre, D.C., 2001. Isotype-specific antibody responses of cattle to *Salmonella* Dublin lipopolysaccharide and porin following *Salmonella* Dublin vaccination and acute and chronic infection. J. Vet. Diagn. Invest. 13, 213-218.
Jensen, A.M., Feld, N., Nielsen, B.B., Schirmer, A.L. and Madsen, E.B., 1994. *Salmonella* Dublin-infektion. (Eradication trial of *Salmonella* dublin infection in 8 dairy herds). Dan. Veterinærtidsskr. 77, 397-403.
Jensen, A.M., Kjeldsen, A.M. and Alban, L., 2004. Control of *Salmonella* Dublin in 6 Danish dairy herds. A case study. (Sanering for *Salmonella* Dublin i 6 malkekvægsbesætninger. En case-undersøgelse.). Dan. Veterinærtidsskr. 87, 26-36.
Lomborg, S.R., 2006. Immune Suppression of Cattle Suspected as Carriers of *Salmonella* Dublin. Veterinary Master Thesis. The Royal Veterinary and Agricultural University.
Lomborg, S.R., Nielsen, L.R., 2006. Immune Suppression of Cattle Suspected as Carriers of *Salmonella* Dublin. In: Proceedings of the International Symposium Salmonella and Salmonellosis. Ploufragan, France.
Nielsen, B.B., Vestergaard, E.-M., 1992. Use of ELISA in the eradication of *Salmonella* Dublin infection. *In*: Proceedings of the International Symposium on Salmonella and Salmonellosis., pp. 220-224.
Nielsen, L.R., 2003. *Salmonella* Dublin in dairy cattle: Use of diagnostic tests for investigation of risk factors and infection dynamics. PhD Thesis. The Royal Veterinary and Agricultural University.
Nielsen, L.R., Ersbøll, A.K., 2004. Age stratified validation of an indirect *Salmonella* Dublin serum ELISA for individual diagnosis in cattle. J. Vet. Diagn. Invest. 16, 205-211.
Richardson, A., 1973. The Transmission of *Salmonella* dublin to Calves from Adult Carrier Cows. Vet. Rec. 92, 112-115.
Richardson, A., 1974. *Salmonella* Dublin infection in cattle. Aust. Vet. J. 50, 463-466.
Smith, B.P., House, J.K., Dilling, G.W., Roden, L.D. and Spier, S.J., 1992. Identification of *Salmonella* dublin Carrier Cattle. *In*: Proceedings of the International Symposium Salmonella and salmonellosis. Zoopôle, Ploufragan, France., pp. 225-230.
Smith, B.P., Oliver, D.G., Singh, P., Dilling, G., Marvin, P.A., Ram, B.P., Jang, L.S., Sharkov, N. and Orsborn, J.S., 1989. Detection of *Salmonella* dublin mammary gland infection in carrier cows, using an ELISA for antibody in milk or serum. Am. J. Vet. Res. 50, 1352-1360.
Sojka, W.J., Thomson, P.D. and Hudson, E.B., 1974. Excretion of *Salmonella* dublin by Adult Bovine Carriers. Br. Vet. J. 130, 482-488.
Spier, S.J., Smith, B.P., Cullor, J.S., Olander, H.J., Da Roden, L. and Dilling, G.W., 1991. Persistent Experimental *Salmonella* dublin Intramammary Infection in Dairy Cows. J. Vet. Int. Med. 5, 341-350.
Spier, S.J., Smith, B.P., Tyler, J.W., Cullor, J.S., Dilling, G.W. and Da Pfaff, L., 1990. Use of ELISA for detection of immunoglobulins G and M that recognize *Salmonella* dublin lipopolysaccharide for prediction of carrier status in cattle. Am. J. Vet. Res. 51, 1900-1904.
Veling, J., 2004. Diagnosis and control of *Salmonella* Dublin infections on Dutch dairy farms. PhD Thesis. Animal Health Service, Deventer, The Netherlands.

Interferon-gamma testing for exposure to paratuberculosis in heifers

Gregers Jungersen, Susanne N. Grell, Anders Stockmarr

Technical University of Denmark, National Veterinary Institute, Dept. of Veterinary Diagnostics and Research

ABSTRACT
The possibilities to develop and refine a test for measurement of paratuberculosis specific cell-mediated immune responses in blood samples have been investigated. Although many improvements have been made it is also clear that there are many inherent problems with this type of test compared with serological tests. Identified problems are low specificity for individual samples (which may be improved with more well-defined antigens) and varying levels in individual IFN-γ response in samples collected from week to week. In addition there is a requirement of culturing the samples with antigens at the earliest possible time point, a problem that has been solved by the addition of the co-stimulatory cytokine IL-12. Although the IFN-γ test is far from perfect, and not yet in a state where results can be made available to farmers, we believe this test is the best current option to provide an independent test supplement to serological analyses. This will become necessary in future stages of a national paratuberculosis eradication program, when assessments of paratuberculosis exposure to young stock are needed in herd classifications.

INTRODUCTION
Serological tools for early diagnosis of paratuberculosis are severely impaired by a lack of sensitivity and specificity. The sensitivity is low because only animals with progressive paratuberculosis develop antibodies that are measurable by the commonly used absorbed ELISA tests. Specificity is low because antigens are cross-reactive with other bacteria. As described elsewhere in these proceedings, the sensitivity increases as the animals become older and start excreting bacteria to the environment.

The pathogenesis of paratuberculosis involves intracellular growth of the bacteria in macrophages. At this hidden location the bacteria are inaccessible for antibodies, hence antibodies are produced in low amounts, and an effective immunological reduction in bacterial numbers is dependent on a strong cell-mediated immune response (CMI). The CMI response involves generation of antigen specific helper T-cells which provide a positive feed-back signal to the infected macrophage to activate the intracellular killing mechanisms of the macrophage. The most important factor in this T-cell mediated feed-back is production of interferon-gamma (IFN-γ). Quantitative measurements of (CMI) responses by the IFN-γ test is gaining increasing acceptance in diagnosis of bovine tuberculosis, brucellosis and paratuberculosis, but still needs further technical improvements and field evaluations to be used rationally in an eradication campaign.

The diagnostic IFN-γ test involves incubation of live blood cells with relevant antigens. During culture, white blood cells in the sample cross-talk with an active presentation of co-cultured antigens by monocytes and recognition of antigens by T-cells. Upon termination of the whole blood culture, IFN-γ secreted into the culture media by antigen responsive T-cells can be measured by an ELISA protocol and the level of IFN-γ is interpreted as a measure of the paratuberculosis specific CMI of the animal. One of the inherent problems with this assay is that time elapsed from collection of the blood sample to start of culture, influences the viability of the cells. Therefore it is recommended to start culture within 8-12 hours of sampling to obtain valid results (Rothel et al., 1992; Jungersen et al., 2002; Robbe-Austerman et al., 2006). Such a protocol is, however, incompatible with delivery of samples by ordinary mail, and it was the aim of the current project to investigate possibilities to make the IFN-γ test more practically feasible. This could be done as an on-site stimulation and incubation in mastitis incubators at the local veterinarian, or by providing the immune cells with recombinant co-stimulatory factors to revive cell functions in day-old samples. Furthermore, it was the objective to evaluate the possibility to use the IFN-γ test as a tool in the surveillance of the herds with an ongoing paratuberculosis eradication scheme and provide the earliest possible feed-back to the farmer on the success of his interventions.

RESULTS
Following early experiments with freeze dried antigens in blood containers and direct addition of freshly collected blood with a syringe and needle, it was quickly decided that such a protocol was not feasible in the long run. We then hypothesized that preformed IFN-γ might be released momentarily if co-stimulatory cytokines were present along with the freeze-dried antigens. We were, however, not able to verify this and found that cultures had to be incubated at 37C for several hours for a measurable production of IFN-γ.

After these and other initial experiments as described in the 8ICP Proceedings (Jungersen et al., 2005) we finally decided upon a protocol with collection of whole blood in heparinized vacutainer tubes, postal freight service and culture on the following day with addition of the co-stimulatory cytokine interleukin-12 (IL-12). IL-12 is known to regulate T_{H1} type immune responses and enhance IFN-γ responses to antigenic stimulation in many animals including bovines (Collins et al., 1998; Collins et al., 1999). We were able to obtain recombinant bovine IL-12 through a collaboration with Chris Howard and Jayne Hope at The Institute

of Animal Health in Compton UK. Fig. 1 shows results from a single vaccinated animal and the effect of blood sample overnight storage temperature on the specific (PPD) and control stimulated (SEB or PBS) IFN-γ production with or without addition of IL-12 in culture.

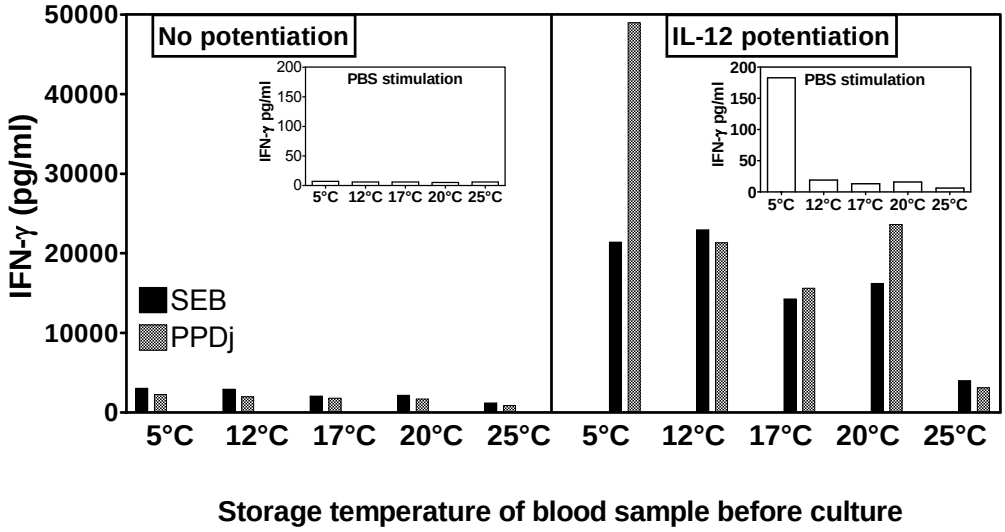


Fig. 1. Effect of storage temperature of blood sample on subsequent IFN-γ production in culture. Blood samples from a stud vaccinated against paratuberculosis were kept over night at 5, 12, 17, 20 and 25ºC before performance of the IFN-γ test. SEB is Staphyloccocus enterotoxin B, a superantigen able to induce IFN-γ without antigen recognition. PPD is paratuberculosis specific Purified Protein Derivative and PBS is Phosphate Buffered Saline as negative media control.

The selected protocol was used on blood samples collected from heifers in the DubPar intervention herds from October 2004 with 1 or 2 annual samplings in spring or autumn. Since we were unable to validate the test we decided not to provide the results to the farmers as we could not yet supply any key for solid interpretation.

Previous reports on the use of the IFN-γ test have not investigated the level of variation in response to repeated sampling in a short period of time. We therefore collected whole blood samples from 25 heifers in the 15-26 month age group from a farm with known problems with clinical paratuberculosis. The samples were stimulated with or without IL-12, and on the day of collection or the following day. These results verified that addition of IL-12 rescues a waning IFN-γ response in day-old samples, but also indicated that addition of IL-12 in some cases changes the interpretation of a given animal. We were not able to verify which results most correctly reflected the immune status of the animal. The “validation” also showed that a significant difference in the IFN-γ production could be observed in blood samples from a single animal from week to week. We concluded that the IFN-γ test is not suitable for individual diagnosis of animals, but may be a valuable tool for the measurement of previous or current exposure to paratuberculosis bacteria in a group of animals.

We also observed very high variation in the level of IFN-γ in response to the SEB positive control culture. Since the SEB response should reflect the ability of the sample to respond to antigenic stimulation, this observation might influence the interpretation of paratuberculosis-specific responses in samples collected from the study herds. To take the level of IFN-γ production of both positive and negative control stimulations into account for interpretation of an individual sample, a mathematical algorithm was produced. However, the lack of a well-defined negative herd among the DubPar intervention herds was apparent, and the current status is that we will use the Danish Operation Paratuberculosis to identify candidate herds for negative control herds, and then hopefully validate interpretation of the test results of the many samples we have gathered in the DubPar project. The current situation is therefore that no results of the heifer screenings are provided to the participating farmers. This is most unfortunate, but we do not want to risk erroneous conclusions from results that may not be as useful as we originally hoped them to be.

DISCUSSION

As the level of paratuberculosis exposure is expected to decrease in the next years as a result of the Operation Paratuberculosis it will be increasingly important to be able to certify herds without serological reactors as free of the disease. The currently available serological tests do not allow for any certification of young animals and it will therefore be important to test heifers and other young stock by techniques exploring the early cell-mediated immune responses against paratuberculosis. Currently, the IFN-γ test is the best

option for this certification and pre-trade screening. However, more research is needed in terms of better antigens, valid interpretation and statistical analysis of the data collected in this project. Some of this work will be included in a new collaborative PhD study with the National Veterinary Institute, The Faculty of Life Sciences and Danish Cattle Organisation.

It is important to keep in mind that, at least with currently available antigens, paratuberculosis specific IFN-γ responses do not discriminate animals with an efficient CMI response clearing the mycobacterial infection from animals with a subclinical progressive chronic infection. During an eradication campaign different strategies for these types of animals may be desired: In herds with low incidence of paratuberculosis both types of animals should be culled, but in high incidence herds, the latter group should be selectively identified and handled according to the outlined plan for risk animals. It is under no circumstances an advantage to make a selective pressure on the animals with the highest IFN-γ responses if these are not truly reflecting a difference in paratuberculosis susceptibility, but merely are reflecting differences in e.g. genetic potential to respond to PPD antigens. Therefore, we find it essential to perform a thorough validation of this new test before it is used in the current strategies to combat paratuberculosis in Denmark.

In recent years microarray studies have allowed the simultaneous analysis of a wide range of host response genes in animals infected with *M. avium* subsp. *paratuberculosis*. Such analyses are not yet complete but there are indications that immune response parameters other than IFN-γ (e.g. apoptosis) may reliably predict an inefficient immune response to *M. avium* subsp. *paratuberculosis* infection. The results and experiences in the current project will be pursued in the future in an attempt to employ these immune response parameters in an antigen specific blood test that can supplement or replace the IFN-γ test and provide further information on which direction the infection will take in the individual animal.

REFERENCES

Collins, R.A., Camon, E.B., Chaplin, P.J., Howard, C.J., 1998. Influence of IL-12 on interferon-gamma production by bovine leucocyte subsets in response to bovine respiratory syncytial virus. Vet. Immunol. Immunopathol. 63, 69-72.

Collins, R.A., Howard, C.J., Duggan, S.E., Werling, D., 1999. Bovine interleukin-12 and modulation of IFN-gamma production. Vet. Immunol. Immunopathol. 68, 193-207.

Jungersen, G., Grell, S.N., Clemensen, A., Roust, T., Howard, C.J. Interleukin-12 potentiation of the interferon-gamma test rescues day-old blood samples for the diagnosis of paratuberculosis. *In* : Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005, p. 501-506.

Jungersen, G., Huda, A., Hansen, J.J., Lind, P., 2002. Interpretation of the gamma interferon test for diagnosis of subclinical paratuberculosis in cattle. Clin. Diagn. Lab. Immunol. 9, 453-460.

Robbe-Austerman, S., Krull, A.C., Stabel, J.R., 2006. Time delay, temperature effects and assessment of positive controls on whole blood for the gamma interferon ELISA to detect paratuberculosis. J Vet. Med B Infect. Dis. Vet. Public Health 53, 213-217.

Rothel, J.S., Jones, S.L., Corner, L.A., Cox, J.C., Wood, P.R., 1992. The gamma-interferon assay for diagnosis of bovine tuberculosis in cattle: conditions affecting the production of gamma-interferon in whole blood culture. Aust. Vet. J. 69, 1-4.

Modelling transmission of *Salmonella* Dublin

Liza Rosenbaum Nielsen[1], David Jordan[2],Gerdien van Schaik[3], and Anne Braad Kudahl[4]

[1]*University of Copenhagen, Faculty of Life Sciences (LIFE), Dept. of Large Animal Sciences;*
[2]*New South Wales Primary Industries, Wollongbar, NSW, Australia;*
[3]*Animal Health Service, Deventer, The Netherlands;*
[4]*University of Aarhus, Faculty of Animal Sciences*

ABSTRACT
The impact of strategies for controlling a disease can be assessed by either gathering empirical evidence from observational or intervention studies in the field or by constructing models that mimic the pathogen's behaviour in the population and environment. Both approaches have advantages and disadvantages. Modelling the ecology of a pathogen is useful for developing a simplified understanding of the behaviour of the infection. Models can highlight those features of the infection that are best targeted by interventions and enable scrutiny of the effectiveness of these strategies under different scenarios.

Several models of *Salmonella* infection dynamics have been reported in the literature. After a short introduction to the concept of modelling infectious diseases we summarise the use of these models and the most important results and conclusions from these studies.

Controlling or eradicating *Salmonella* (*S*.) Dublin from cattle herds requires intervention by closing transmission routes and minimizing the exposure to the bacteria in the environment or from other infectious animals. Models have helped to identify the critical aspects of the dynamics of *S.* Dublin infection in infected herds. For instance, they point to the need to know more about the relative effect of contaminated environment, and the relationship between infectious animals, infectious herds and the infection load in the environment. Also, it is still uncertain as to what extend it is necessary to cull persistently infected animals from the herd in order to eradicate the infection from the herd. The different studies are somewhat contradictory with regards to these issues, however, most agree that the environmental sources of infection are important for both within-herd and between-herd transmission and thus are important to control.

Infection dynamics and the effect of intervention strategies will be studied further in a new *S.* Dublin-specific model to be implemented in the PTB-SimHerd-model developed at the Faculty of Animal Sciences, Foulum in Denmark. Experiences from intervention trials and previous modelling of *Salmonella*-infections in cattle will be used to construct this model. As transmission routes for *S.* Dublin and *Mycobacterium avium* subsp. *paratuberculosis* (causing paratuberculosis) are almost identical, the simultaneous effects on both diseases of closing these routes can be estimated by this model.

WHY MODEL *SALMONELLA*?

Modelling the dynamics of an infection is trying to find a simplistic way to illustrate the behaviour of the infection as a way to obtain better understanding of important features of the infection. Often, building the model and performing studies to fill in missing pieces in the model lead to new understanding of the infection pathogenesis or epidemiology that one would not have obtained by looking at a particular hypothesis as an isolated event. For instance, quite a few studies have been performed to obtain better knowledge about the pathogenesis, detection and risk of shedding of bacteria from persistently infected carrier animals (Richardson, 1973; Smith et al., 1989; Spier et al., 1990; Spier et al., 1991; Smith et al., 1992; Hoorfar et al., 1996; Nielsen et al., 2004; Lomborg and Nielsen, 2006), but only rarely has the effect of removing or handling suspected carrier animals from infected herds been studied (Veling, 2004; Bergevoet R.H.M. et al., 2006), and more work is needed to demonstrate the importance of this intervention strategy under different scenarios.

The effect of intervention strategies usually has to be assessed either through intervention studies in the field or with models that mimic the reality. Both approaches have advantages and disadvantages. Field studies are expensive and time consuming. It is often difficult to study the effect of single intervention strategies, because the farmer may not want to do just one thing but may change several management strategies when trying to control *S*. Dublin. The more single intervention strategies that one wants to investigate in a field study the more herds it requires. There should be sufficient number of herds in the study to take into account other risk factors for the infection such as herd size, barn type, geographic location, grassing practices etc. (Vaessen et al., 1998; Fossler et al., 2005a; Fossler et al., 2005b; Nielsen et al., 2006). It quickly becomes too overwhelming and expensive to perform such a study. For example, the cost of collecting specimens and having them processed in a laboratory is often so prohibitive that it limits the ability of investigators to design a study that has any hope of meeting useful objectives. Further, it may be difficult to find proper control herds, because once you start talking about the infection most farmers will become interested and may start to do something – for instance cleaning out the calving area and removing calves quickly from the dam after birth. However, field studies are necessary to obtain practical experience

with solutions in the herds and learning about what is feasible under different circumstances in the herds. Field studies often provide crucial information to pieces of the models.

Modelling the infection may be an alternative to field studies or at least a reasonable supplement to intervention field studies. In models, different elements of importance for the infection dynamics and intervention strategies can be gathered and set to have an impact on each other. One parameter can be changed to mimic performing a certain intervention task in the herd while keeping the other parameters fixed, or several parameters can be changed simultaneously to represent intervention by a set of recommendations. Some models include herd specifications of known risk factors. This way the model may become more specific for certain types of herds and the results become more relevant for such herds. Some models may simulate what will happen with the infection in the herds or on a national/regional level over time for different scenarios. Some models take into account the fact that *Salmonella* are bacteria that have stochastic expressions, meaning that they do not always act in the same way even when the circumstances are the same. In other words, there is some degree of random variation in the outcome when the infection is introduced and spreads through animals and herds.

TYPES OF WITHIN-HERD AND BETWEEN-HERD *SALMONELLA* TRANSMISSION MODELS

Models of *Salmonella* infection dynamics may be on within-herd level or between-herd level. The within-herd models try to model what happens after introduction of an infectious animal into a fully susceptible herd and some models try to predict the long-term behaviour after the initial spread of the infection. Does the infection become endemic in the herd? What percentage of animals become infected and at the endemic state how many animals on average are susceptible, infectious and immune at any given time.

Between-herd models model the movement of infection between cattle herds and try to illustrate what happens overall with the prevalence of infected herds in a region or country over time, when different risk factors for introduction and persistence of the infection change. For instance, if restrictions are posed on infected herds so that they cannot sell animals to live use how does this change the prevalence over time and will it be sufficient to bring the prevalence to 0%?

Most models for *Salmonella* in cattle work as so-called SIR-models. For within-herd models, all animals in the herd are assigned an infection status: susceptible (S), infectious (I) or recovered (R). Some models are extended to include other possible infection statuses such as latent infection (L) meaning that the animal is infected but not currently shedding bacteria and thus not infectious. Animals can move between these states with a certain rate. Infection rate indicates the number of animals that move from S to I per time unit. Recovery rate indicates the number of animals that move from I to R per time unit. Most *Salmonella* models also allow the animals to loose immunity after a while. This is supported by experimental and field studies (Steinbach et al., 1996; Nielsen, 2003). Thus, the immunity loss rate should also be included in the model.

The time unit chosen depends mostly on what is believed to be true for the infection course in the individual. Ideally the time unit should be short enough that only one new generation can become infectious from the time the previous generation of animals became infectious. This is highly dependent on the incubation period, which for *Salmonella* is believed to be very short, probably only 1-2 days. Experimentally, animals begin to excrete bacteria already 12-48 hours after they become inoculated (Robertsson, 1984; Segall and Lindberg, 1991). However, transfer of infection between animals not only depends on when the animals are shedding, but also contact patterns between animals.

The principle for between-herd models may be the same as for within-herd models except it is herds not animals that are assigned infection statuses. One major difference is that herds to not move around and therefore the contact patterns between herds are very governed by their location and purchase behaviour.

As it will become evident in the following presentation of different *Salmonella* models and the conclusions from these model, including environmental components that allow for new infections to occur even when there are no infectious animals present in the group of animals under study seems to be important for *Salmonella* models to work. Likewise, new infections arising from environmental sources seem to be important for transmission between cattle herds, though movement of cattle between herds is still the main risk for introduction of infection to a herd. The between herd models also point to the importance of reducing the infectious period in herds that become infected. Within-herd models are probably the best to show how this can be done. Thus, both types of models are important and combining them successfully could reach a new level of complexity and lead to better understanding of the infection dynamics of *Salmonella* within a country or region.

Most of the models are used to estimate an important parameter that characterises the infection under different scenarios and that can be used to compare the effect of changing the scenarios through intervention strategies, such as lowering the probability of transmission of bacteria between neighbouring calves in the barn. This parameter is called the basic reproduction number or R naught (R_0). R_0 is defined as the average number of secondary cases produced in a completely susceptible population, by a typical infected individual, during its entire period of infectiousness (Anderson and May, 1991). Under the assumption of homogenous mixing of susceptible individuals, the higher R_0 is, the higher is the probability

that the infection will invade the group of susceptible individuals when it is introduced and the resulting size of the epidemic (how many animals become infected) also depends on R_0. The subsequent course of the infection depends both on R_0 and the population dynamics, i.e. group size, natural influx to and departure from the group, case-mortality rates and loss of immunity-rate. If R_0 can be reduced to markedly below 1 by intervening in the herd, the probability of the infection producing epidemics or becoming endemic in the herd upon introduction of an infectious individual is low or non-existing. Thus, R_0 can be used to compare the efficiency of different strategies, besides giving a more general idea about the nature of the infection.

Estimation of R_0 for S. Dublin in young calves based on Danish field data (Nielsen et al., 2007)

In the project "Diagnosis, disease dynamics and intervention: *S.* Dublin and paratuberculosis", we have used data collected during the socalled "Kongeå-project" (Andersen et al., 2000) to estimate infection parameters of *S.* Dublin among young calves. Antibody measurements (ELISA) and bacteriological culture (BC) data collected weekly over a period of 16 weeks in 2001-2 were used to estimate number of days of faecal excretion of *S.* Dublin bacteria and time to seroconversion in infected calves below the age of 180 days. Based on these estimates and the literature, all calves in four endemically infected dairy herds were grouped into the following infection states: susceptible (S), infectious (I) and resistant/recovered (R) as illustrated in Fig. 1.

Estimates of transmission parameter, β, were obtained from a generalised linear model relating the number of new infections to the proportion of susceptible and infectious calves per week. From β, the basic reproduction ratio, R_0, was estimated for each herd and across herds. The point estimates for R_0 ranged from 1.1 to 2.7 in the study herds. However, the confidence intervals were wide. Data were too limited to show possible significant differences in the parameters between the study herds. However, the tendency in the data suggested that there may be important differences. Across herds, the R_0 was close to 2 suggesting that on average one infectious calf will produce two new infectious calves when introduced into a fully susceptible population under typical Danish dairy production systems. The model was not able to run without including an environmental component that allowed for infections to occur in weeks where no infectious animals were present according to the classification criteria. The analyses indicated that environmental contamination from infectious calves plays an important role in transmitting *S.* Dublin between calves.

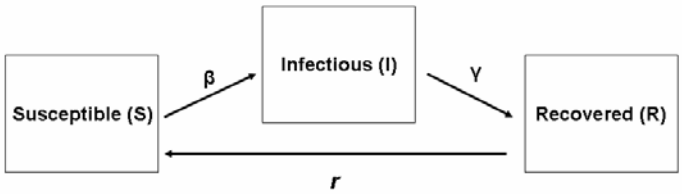


Fig. 1 Schematic presentation of an SIR-model of infection dynamics of *S.* Dublin among young calves in dairy herds (*β*=transmission rate, *γ*=recovery rate, *r*=immunity loss rate). Modified from Nielsen et al. (2007).

The good thing about this type of model is that it is fairly easy to perform once the data has been collected and that it is based on real life data from typical Danish dairy herds infected with *S.* Dublin, which gives credit to the reliability of the results for people with limited or no previous exposure to mathematical modelling. The classification of the calves were probably too deterministic in the sense that we used average durations of infectiousness in stead of allowing for more random or individual variations in the duration of shedding and the time to seroconversion from introduction of infection to each calf. Stochastic type of models will allow for such variations to be included in the model and should be considered, in particular for small group sizes of calves as found in dairy herds in Denmark.

Estimation of R_0 for Salmonella based on Dutch field data (van Schaik et al., 2007)

In general, cattle in the Netherlands are infected with two types, *S.* Dublin and *S.* Typhimurium. Both types cause clinical symptoms *S.* Dublin but outbreaks are more prevalent and more clinical than *S.* Typhimurium outbreaks. Our knowledge of the transmission of *Salmonella* within herds is still limited, while this is an essential component for modelling the success of intervention strategies to control *Salmonella*. The aim of the Dutch study was to estimate the basic reproduction ratio (R_0), the number of secondary cases produced from each primary case in a totally susceptible population, for *S.* Dublin and *S.* Typhimurium in dairy herds.

Serological data were obtained from eight farms with a clinical outbreak of *Salmonella*, two with an outbreak of *S.* Dublin and six of *S.* Typhimurium. R_0 was estimated from the serological data of the herds that were in an endemic state of the infection. For *Salmonella* dynamics the population of size *N* can be divided in four compartments: susceptible animals (*S*), latently infected animals (*L*), infectious animals (*I*),

and seropositive animals (recovered, *R*). The change in the numbers of animals in the four compartments is described by differential equations. In an endemic situation, the average number of animals in each class does not change in time, so all differential equations can be set equal to 0. R_0 can than be derived from the fraction of seropositive animals (*R*/*N*) in the endemic situation (Anderson and May, 1991).

R_0 across herds was estimated to be 2.5 (95%CI 1.7-9.8) and 1.3 (95% CI 1.1-1.7) for *S.* Dublin and *S.* Typhimurium, respectively. The between herd variation was significant and fairly large. The results of the sensitivity analysis showed that the R_0 estimate was not sensitive for changes in the latent, infectious or seropositive periods. The R_0 estimates indicated that the infection would not spread very extensively in susceptible populations under management systems similar to the ones in the study herds.

Mathematical model of within herd infection dynamics of Salmonella (Xiao et al., 2005)

The model developed in this study provided a mathematical framework for understanding of *Salmonella* infections with different characteristic in dairy herds including *S.* Dublin.

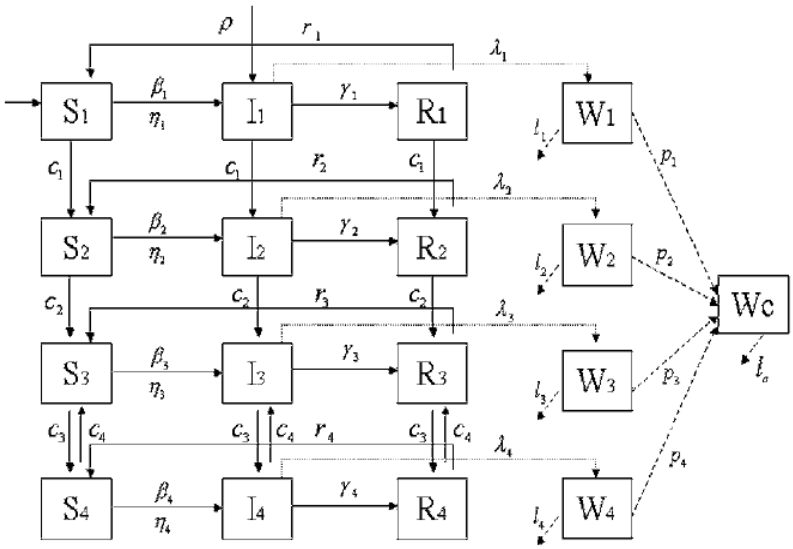


Fig. 2 Flow diagram of transmission routes and population dynamics in dairy herds with four groups of cattle (1=preweaned calves, 2=weaned heifers, 3=dry cows and in calf heifers, 4=lactating cows). S=susceptible, I=infectious, R=recovered (immune) and W=four local and one common environmental compartments that receive free-living bacteria from infectious animals and via common equipment and affect the rate of infection of susceptibles. *β*=direct transmission rate, *γ*=recovery rate, *r*=immunity loss rate, *λ*=shedding rate, *p*=pooling rate, *c*=maturation rate of the animals (or rate of moving between groups in the barn), *ρ*=pseudo-vertical transmission rate, l=death rate of organism. All rates were given per day. (Reprintet from Journal of Theoretical Biology, 233, 159-175, Understanding the dynamics of *Salmonella* infections in dairy herds: a modelling approach, Xiao et al., 2005 with permission from Elsevier).

The model frameworks was build up as deterministic SIR-models in each of four groups in the herd and animals could the move between these four groups as illustrated in Fig. 2. In this study, the effect of changing several different parameters in the model on the resulting R_0 and the epidemic size, peak and subsequent persistence of infection was used to demonstrate the importance of different intervention strategies and features of *Salmonella* infections including the effect of culling strategies.
Some of the main conclusions were that 1) pseudovertical transmission (from dam to calf) did not appear to have significant effect on introduction and persistence of *Salmonella* infections, however the authors did not specify whether it could be important in the situation with persistently infected carrier animals; 2) indirect transmission via the group-specific (local) environmental compartments were important for introduction of infection, in particular measures aimed at reducing indirect transmission should be concentrated on the lactating group environment; 3) recovery rate, pathogen-induced mortality, immune response and pathogen removal rate were found to be factors inducing small fluctuations of epidemics after the initial introduction, however, it was not specified which sets of parameters would produce patterns consistent with typical *S.* Dublin-expressions in the herds. These are desirable perspectives to look into using this model; 4) prevalence of infection in the lactating group is higher than that in other groups. This is contradictory to what we have found in the study concerning detection of *S.* Dublin shedding animals (see “Challenging the traditional methods for detection of *Salmonella* Dublin carrier animals” elsewhere in these proceedings). These contradictory findings may be due to differences in serotypes studied and it is likely that the model by Xiao et al. (2005) is more relevant for other serotypes, such as Typhimurium. The model did not include

seasonal changes in parameters. This is probably important to include as seasonality is a well-known factor influencing the prevalence of infection in all species.

Decision support model to evaluate Salmonella control strategies in the Netherlands (Bergevoet R.H.M. et al., 2006)

A *S.* Dublin and Typhimurium control programme is considered in the Netherlands. *Salmonella* control strategies were evaluated using a decision support model consisting of an epidemiological and an economical part. The epidemiological model was a state transition model. The unit of analysis was the individual farm and the infectivity of a herd for other herds was considered. Interventions were modelled as influencing the impact of risk factors on the transmission of *Salmonella* in the model.

Different voluntary as well as obligatory strategies were defined. Amongst the interventions were prohibition of movement of potentially infectious animals and manure, culling of chronically infected animals to reduce infectivity and length of infection in the herds, and herd management measures such as separate housing of age-groups. Reduction of prevalence of infected herds, cost of the strategy for the dairy sector, and cost effectiveness were calculated for each strategy.

Results of the model suggested that obligatory strategies, which included four-monthly bulk-milk sampling to determine and monitor the status of the herd, prevention of movement of potentially infected animals and the culling of chronically infected cattle from the herds were able to reduce the prevalence of test positive herds considerably and while being most cost effective. It was also found that after four years of similar intervention strategies the effect on prevalence levelled out and a steady state with 3-4% infected herds was reached.

A virtual herd approach to modelling transmission and control of Salmonella Dublin (current EpiLab-project)

A model currently being developed in a collaborative International EpiLab-project is a model that departs from the traditional approach by placing less reliance on mathematical processes and more emphasis on statements of logic that summarise the behaviour of elements in the system as understood by biologists. Reliance is placed on object-oriented computer code to create a hierarchical structure of animals, herds, and regions that mimics the Danish dairy industry. The traditional three compartment (SIR) approach to disease modelling is replaced by an infection-recovery cycle for herds that has five states describing combinations of traits relevant to the spread and control of *S*. Dublin.

Superimposed on this is a control programme based on recurrent testing of bulk tank milk combined with classification of herds according to risk of infection (Level status), and a system of exposing clean herds to new infection to mimic the movement of livestock between herds and the purchasing practices of herd managers. Inputs that define aspects of herd infection, herd recovery, livestock trading practices, and features of the surveillance scheme can be made both stochastic and optionally dependent on the geographic region in which a herd is located.

Effectively the model creates herds and regions, assigns them attributes relevant to the transmission of *S*. Dublin and follows each herd through time. The status of each attribute of each herd is updated with each time step in the model. With this approach it is possible to generate informed expectations of progress in the control of *S.* Dublin in the Danish cattle industry over a five to ten year period. Advantages of a model that 'is more biological than mathematical' include its intuitive appeal to researchers and policy makers and a capacity to assimilate a greater proportion of the findings from research and surveillance.

REFERENCES

Andersen, H.J., Aagaard, K., Skjøth, F., Rattenborg, E. and Enevoldsen, C., 2000. Integration of research, development, health promotion, and milk quality assurance in the Danish Dairy Industry. *In*: Salman, M.D., Morley, P.S., Ruch-Galie, R. (Eds.), Proceedings of the 9th Symposium of the International Society of Veterinary Epidemiology and Economics (ISVEE9), Colorado, 2000, pp. 258-260.

Anderson, R.M., May, R.M., 1991. Infectious Diseases of Humans: Dynamics and Control. Oxford University Press, New York.

Bergevoet R.H.M., van Schaik, G., Veling, J., Backus G.B.C. and Franken, P., 2006. Economic and epidemiological evaluation of possible *Salmonella* control strategies in dairy cattle. *In*: Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, Cairns, 2006.

Fossler, C.P., Wells, S.J., Kaneene, J.B., Ruegg, P.L., Warnick, L.D., Bender, J.B., Eberly, L.E., Godden, S.M. and Halbert, L.W., 2005a. Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms: I. *Salmonella* shedding in cows. Prev. Vet. Med. 70, 257-277.

Fossler, C.P., Wells, S.J., Kaneene, J.B., Ruegg, P.L., Warnick, L.D., Bender, J.B., Eberly, L.E., Godden, S.M. and Halbert, L.W., 2005b. Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms: II. *Salmonella* shedding in calves. Prev. Vet. Med. 70, 279-291.

Hoorfar, J., Wedderkopp, A. and Lind, P., 1996. Comparison between persisting anti-lipopolysaccharide antibodies and culture at postmortem in salmonella-infected cattle herds. Vet. Microbiol. 50, 81-94.

Lomborg, S.R., Nielsen, L.R., 2006. Immune Suppression of Cattle Suspected as Carriers of *Salmonella* Dublin. *In*: Proceedings of the International Symposium *Salmonella* and Salmonellosis.,

Nielsen, L.R., 2003. *Salmonella* Dublin in dairy cattle: Use of diagnostic tests for investigation of risk factors and infection dynamics. PhD Thesis. The Royal Veterinary and Agricultural University.

Nielsen, L.R., Schukken, Y.H., Grohn, Y.T. and Ersbøll, A.K., 2004. *Salmonella* Dublin infection in dairy cattle: Risk factors for becoming a carrier. Prev. Vet. Med. 65, 47-62.

Nielsen, L.R., Warnick, L.D. and Greiner, M., 2006. Risk Factors for Changing Classification Level in the Danish *Salmonella* Surveillance Program for Dairy Herds. *In*: Proceedings of the 11th International Symposium of Veterinary Epidemiology and Economics (ISVEE), Cairns, 2006, no. 511

Nielsen, L.R., van den Borne, B. and van Schaik, G., 2007. *Salmonella* Dublin infection in young dairy calves: Transmission parameters estimated from field data and an SIR-model. Prev. Vet. Med. 79, 46-58.

Richardson, A., 1973. The Transmission of *Salmonella* dublin to Calves from Adult Carrier Cows. Vet. Rec. 92, 112-115.

Robertsson, J.A., 1984. Humoral antibody responses to experimental and spontaneous *Salmonella* infections in cattle measured by ELISA. Zentralbl. Veterinarmed. B. 31, 367-380.

Segall, T., Lindberg, A.A., 1991. Experimental oral *Salmonella* dublin infection in calves: A bacteriological and pathological study. J. Vet. Med. B. 38, 169-184.

Smith, B.P., House, J.K., Dilling, G.W., Roden, L.D. and Spier, S.J., 1992. Identification of *Salmonella dublin* Carrier Cattle. *In*: Proceedings of the International Symposium Salmonella and salmonellosis. Zoopôle, Ploufragan, France, 1992, pp. 225-230.

Smith, B.P., Oliver, D.G., Singh, P., Dilling, G., Marvin, P.A., Ram, B.P., Jang, L.S., Sharkov, N. and Orsborn, J.S., 1989. Detection of *Salmonella dublin* mammary gland infection in carrier cows, using an ELISA for antibody in milk or serum. Am. J. Vet. Res. 50, 1352-1360.

Spier, S.J., Smith, B.P., Cullor, J.S., Olander, H.J., Da Roden, L. and Dilling, G.W., 1991. Persistent Experimental *Salmonella dublin* Intramammary Infection in Dairy Cows. J. Vet. Int. Med. 5, 341-350.

Spier, S.J., Smith, B.P., Tyler, J.W., Cullor, J.S., Dilling, G.W. and Da Pfaff, L., 1990. Use of ELISA for detection of immunoglobulins G and M that recognize *Salmonella dublin* lipopolysaccharide for prediction of carrier status in cattle. Am. J. Vet. Res. 51, 1900-1904.

Steinbach, G., Koch, H., Meyer, H. and Klaus, C., 1996. Influence of prior infection on the dynamics of bacterial counts in calves experimentally infected with *Salmonella* dublin. Vet. Microbiol. 48, 199-206.

Vaessen, M.A., Veling, J., Frankena, K., Graat, E.A. and Klunder, T., 1998. Risk Factors for *Salmonella* Dublin infection on Dairy Farms. Vet. Quart. 20, 97-99.

van Schaik, G., Klinkenberg, D., Veling, J. and Stegeman, J.A., 2007. Transmission of *Salmonella* in dairy herds quantified in the endemic situation. Vet. Res. Accepted for publication.

Veling, J., 2004. Diagnosis and control of *Salmonella* Dublin infections on Dutch dairy farms. PhD Thesis. Animal Health Service, Deventer, The Netherlands.

Xiao, Y., Bowers, R.G., Clancy, D. and French, N.P., 2005. Understanding the dynamics of *Salmonella* infections in dairy herds: a modelling approach. J Theor. Biol 233, 159-175.

Interpretation of diagnostic test-information for paratuberculosis control and surveillance

Søren Saxmose Nielsen

University of Copenhagen, Faculty of Life Sciences, Dept. of Large Animal Sciences

ABSTRACT
Diagnostic test-information could be useful for making decisions regarding paratuberculosis, depending on whether the information should be used for avoiding further transmission, estimation of production losses, estimation of prevalence of infection etc. Interpretation of test results can be improved, if different conditions are considered. Four conditions are considered in this paper: 1) affected; 2) infectious; 3) infected; and 4) non-infected animals. The interpretation of test-results in relation to each of these conditions is discussed, with a summary regarding the interpretation of the test-results, which can be obtained in the Danish paratuberculosis programme.

INTRODUCTION
Paratuberculosis is a chronic infection, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and predominately affecting cattle and other ruminants. MAP infections may remain latent for the life-span of an animal, but when infections progress, it can lead to significant milk production losses, eventually resulting in emaciation and death of the infected animal.

Infection is usually assumed to take place in calfhood (Doyle, 1953), although it is likely that older animals can also be infected (Larsen et al., 1975). The definition of “infected” in this regard is interesting, in that some animals may show temporary signs of infection such as immune reactions, pathological changes and excretion of MAP. The diagnosis “paratuberculosis” is usually made by detecting the shedding of MAP using faecal culture (FC), immune reactions such as detection of antibodies by use of antibody ELISAs or detection of histopathological changes. Detection of MAP by FC requires that the bacteria are shed in sufficient numbers to be detected. Detection of immune reactions requires that these have occurred. Therefore, when establishing the diagnosis, it is beneficial to have in mind the presumed course of infection. Although there may be major variations, the following is assumed to occur: firstly, the infection is thought to be controlled by a predominating T helper 1 (Th1) response (Stabel, 2000), and MAP can be shed in low numbers in this stage. The next stage of infection is a predominant Th2 response (Stabel, 2000). Both Th1 and Th2 responses will result in production of MAP-specific antibodies, with Th1-responses being characterised by low concentrations of IgG2 and Th2-responses usually characterised by higher concentrations of IgG1. Based on this, the immune responses can therefore be divided into three phases: 1) no immune response; 2) control of infection via Th1-dominated or cell-mediated immune responses; and 3) Th2-dominated humoral immune responses, where control of the infection is appears to be lost.

The diagnostic test information should provide a decision maker with information to make appropriate decisions on the test result. For a decision maker, it is essential to have results, which can be used for appropriate decision making. Consequently, test-evaluations should be performed to support decisions for interpretation of the diagnostic test-information. For primary producers, these decisions may relate to: a) maintaining the herd free of MAP-infections; b) managing infected animals, which may later become infectious or affected; c) managing animals, which are currently infectious; and d) managing affected animals. From this we can derive four categories of animals: A) non-infected; B) infected, but non-infectious; C) infectious; and D) affected, i.e. performance of the animal is reduced (e.g. reduced milk yield, loss of weight).
This paper summarises interpretation of diagnostic test-information related to these four conditions.

DETECTION OF AFFECTED ANIMALS
Detection of affected animals will primarily be of interest in two situations: i) for confirmation that a condition is caused by MAP infection; and b) for prediction of production losses caused by a MAP infection. In the first situation, culling may be instituted at the time of confirmation, whereas in the latter situation, the optimal time of culling would be of interest. These decisions normally pertain only to the individually affected animal, as animal welfare and the actual production of that individual are the parameters of primary interest.

The ability of serum antibody ELISAs to detected affected animals have been included in a few studies only (Bech-Nielsen et al., 1992; Egan et al., 1999; Sweeney et al., 1995). The sensitivities of the tests to detect “clinically affected animals” were reported in the range 0.50 to 0.87. For faecal culture, the sensitivity for detection of affected animals has been reported to. 0.70 (Egan et al., 1999). no concurrent specificity estimates were reported in these studies. Therefore, these estimates could be interpreted as valid for situations where a prior suspicion of MAP infection is presumed, and the positive test-result should be used to confirm the infection.

Simultaneous evaluation of sensitivity and specificity is often desirable, for situations where no prior suspicion of a MAP infection in a specific animal is available. Wang et al. (2006) investigated the entire range of diagnostic values for a milk antibody ELISA and found that the area under receiver operating curves for cows yielding 0 kg energy corrected milk (ECM), 20 kg ECM and 40 kg ECM were 0.86, 0.81 and 0.75, respectively. Their findings indicate that a concurrent drop in milk yield has a significant diagnostic value, and should be included in the interpretation of test-information from milk ELISA.

The predictive value is of primary interest for a decision maker in the situation, where a decision has to be made on a given animal. On average, it has been predicted that Danish Holstein cows with an increase of 1 standardised OD-values had poorer persistency of lactation curves if they were 1st parity cows, and they had a fat corrected test-day milk yield depression of 2.7 kg for the entire 305-day lactation (Kudahl et al., 2004). Another study based on repeated ELISA-testing indicated that cows with repeated positive (>0.3 OD-values) or cows with the last sample positive in ELISA had a milk yield, which was 10 to 12% lower than their herd-mates, irrespective of breed and parity (Nielsen et al., 2006). Therefore, there are indications that milk yield can be used both as a predictor of the MAP-affected status, and as an outcome to assess the production losses following MAP infections. However, there is currently insufficient information to provide solid evidence between the time from occurrence of antibodies to occurrence of production losses, and this association needs to be investigated further, to provide optimal culling decisions regarding affected animals. In this aspect, the infectious risk associated with these animals need to be considered too.

DETECTION OF INFECTIOUS ANIMALS

Infectious animals are a risk for transmitting MAP to susceptible herd mates. Transmission can be blocked if these animals are either culled or isolated from susceptible animals. Multiple studies using antibody ELISA as a diagnostic test to detect infectious animals have been conducted. Due to the vast number of test evaluations reported and the general lack of comparability, we will only report further on the results on the milk ELISA used in the Danish control programme on paratuberculosis.

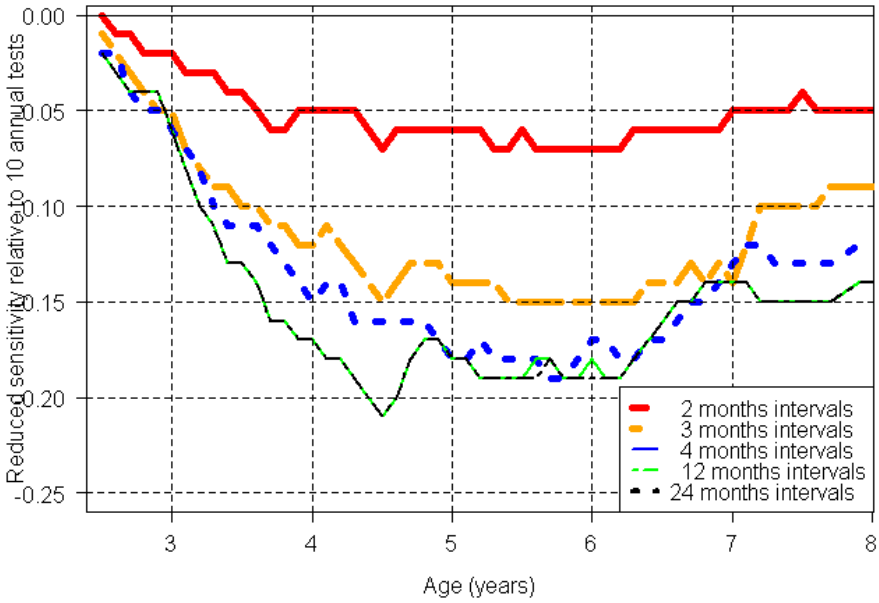


Fig. 1. The effect on the probability of testing positive given that the animals were shedding MAP at different test frequencies. Lines for 12 and 24 months intervals are more of less lying on top of each other.

There have been no previous reports showing that all infectious animals become sero-positive, but in Nielsen and Ersbøll (2006) it was shown that 98% of animals classified as “high-shedders”, and 95% of “low-shedders” were detected by a milk antibody ELISA. The lack of detection of some shedders could be due to cows shedding MAP subsequent to passive uptake of the bacteria from a heavily contaminated environment (Sweeney et al., 1995), lack of detection due to too long interval between tests, or because the animals never sero-converted. In Nielsen and Ersbøll (2006), testing was performed roughly 10 times per year per animal, but the testing scheme did not assure that this average could be maintained for all animals. A high test-frequency will automatically increase the probability of testing positive, potentially producing a significant number of false-positives. However, investigating this further indicated that only animals from 2 to 4 years of age appeared to have a higher probability of testing positive with an increase in no. of tests (Fig. 1). From that analysis, it was also evident that the testing frequency would have a great effect on detection of infectious animals in this age-group. These findings suggest that the number of false-positives do not increase to a great extent due to the high test-frequency. Instead, the results suggest that the increased

number of positives is due to a higher number of animals, which sero-convert although there is no direct evidence to support this conclusion.

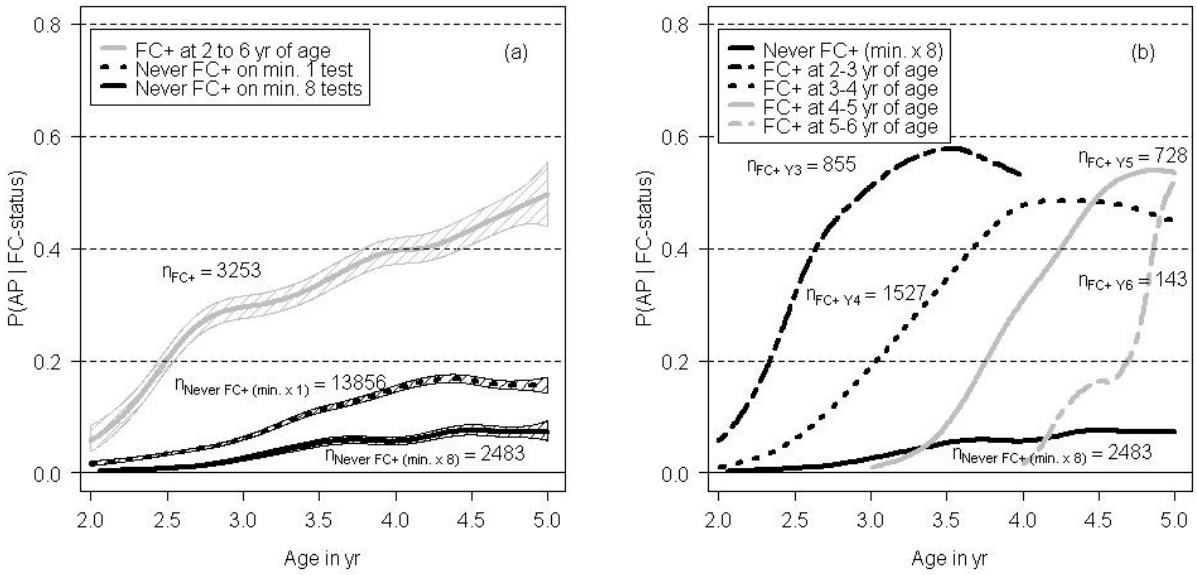


Fig. 2. Estimated probability of testing positive in a milk ELISA for detection of antibodies to *Mycobacterium avium* subsp. *paratuberculosis*, for cows grouped according to the year in which they first tested positive in faecal culture (FC^+). In Figure 2a, the predictions are shown for 3 groups of cows: Never FC^+ (minimum 1 FC), never FC^+ (minimum 8 FC), and FC^+ sometime during the age of 2 to 7 yr. In Figure 2b, the predictions are shown for 5 groups, in which FC^+ cows were divided into groups depending on when they became FC^+ (never; 3rd; 4th; 5th or 6th yr of life).

The time from actually being infectious to becoming ELISA-positive is an interesting parameter, but it has not been investigated in detail. However, in Nielsen and Toft (2006) it was determined that there is a good correlation between age of shedding and age of being ELISA-positive. If animals were tested once per year, the probability of detecting those that became shedders within that year was approximately 50%, irrespective of age (Fig. 2b). If the animals were tested twice per year, this probability apparently increased to 70-90%, but due to sparse data, the uncertainty related to these estimates also increased. Extrapolating from these data and based on the very sparse data remaining for further analyses would suggest that increasing the number of tests per year to 4 would result in the detection of 90 to 95% or more of the shedders. The summarised results are presented schematically in Fig. 3. A rough interpretation would therefore suggest that infectious animals can be detected with 4 annual tests, but some will inevitably be missed. A solution to avoid this situation would be more frequent testing.

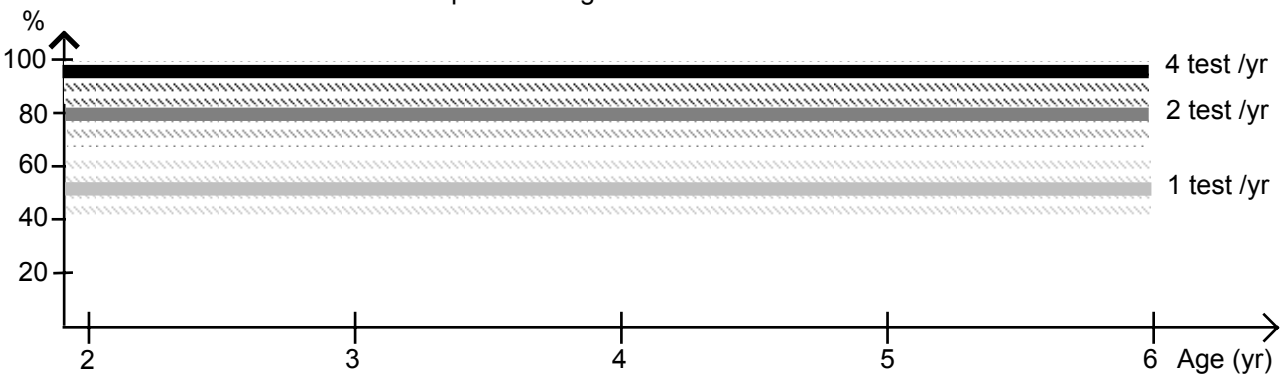


Fig. 3. Schematic presentation of the probability of testing positive in ELISA given the animal is infectious at different ages, for 3 different test-frequencies: 1 test/cow/year; 2 tests/cow/year; and 4 tests/cow/year. The shaded areas represent the uncertainty related to each line.

At the time of decision making, decisions which preclude the ability of infectious animals to transmit bacteria to susceptible herd mates need to be made. The susceptible animals are primarily thought to be calves. Therefore, milk and faeces from the infectious animals should be kept away from calves. Animals that do not get past the infectious stage, may require culling prior to getting into close contact with calves. This situation typically occurs around calving. Any milk from the infectious animals should be used for other

purposes than feeding of animals, which remain in the herd. It is not known when an animal is shedding sufficient bacteria to be considered “infectious” and potentially due for culling. However, the probability of an animal shedding MAP can be derived from the magnitude of the ELISA-reading, where high OD-values indicate a high risk of MAP shedding, and low values indicate a low risk of MAP shedding (Toft et al., 2005). Some non-infectious animals (both non-infected and infected but non-infectious) will also test positive. Therefore, not all test-positive animals should be culled. It is however, important to minimise the risk of transmission from these animals in the period(s), in which they are test-positive.

DETECTION OF INFECTED ANIMALS

Infected animals can carry MAP apparently without being infectious or affected. However, they may at some point in time progress to the next stages of infection. Also, a herd cannot be declared free of MAP infection prior to their removal. Therefore, these animals can on long-term be considered as a risk to further transmit MAP and therefore become an economic burden, but this is not always the case. Therefore, they may constitute a special group of animals, which require decisions of a different nature than that of infectious and affected animals. If detected, they could be culled if the prevalence in the herd is low. At high prevalences, it may be important only to minimise any potential transmission from these animals.

The number of studies, which have investigated the sensitivity and specificity of diagnostic tests used to detect infected animals is limited. For ELISAs, the reported sensitivities are in the range from 0.07 to 0.18, with specificities of 0.91 to 0.98 (McKenna et al., 2005; McNab et al., 1991). Different faecal culture methods have also been evaluated with sensitivities in the range from 0.23 to 0.29 (McKenna et al., 2005; Nielsen et al., 2002; Whitlock et al., 2000). The specificity is difficult to assess, although it is usually assumed to be almost 100%, given that molecular methods are used to confirm the presence of MAP specific DNA-sequences. However, only on herd-level, the test can be assumed to be almost 100% specific. If the test is used in contaminated herds, there is a possibility of obtaining 2% false-positives, i.e. the specificity may be only 98% (Nielsen et al., 2002). False-positive test results could occur as a consequence of the “pass-through” phenomenon, where MAP may be ingested and shed if the animals are housed in heavily contaminated environments, potentially without being infected (Sweeney et al., 1992).

In early stages of MAP infections, the sensitivity of diagnostic tests is low, which could be results of latency of infections, but when progression of infection occurs, the sensitivity should increase. Therefore, age of the animal can be a significant covariate to include, when test-evaluations are performed, because older animals are more likely to have progression of the infection that younger animals. The sensitivity and specificity at different ages was investigated in Nielsen and Toft (2006), and indicated that the sensitivity increased from 0.06 at 2 years of age to 0.50 at 5 years of age (Fig. 2a). In the same age-interval, the specificity appeared to drop from 0.997 to 0.93. This drop in specificity needs to be investigated further, but the drop may be an indication that cows housed in heavily contaminated environments can be transiently infected and clear the infection. Occurrence of transient infections is still speculative, but the apparently low specificity in older age-groups is a challenge in the interpretation of test-results.

DETECTION OF NON-INFECTED ANIMALS

Non-infected animals are per definition the opposite of infected animals. However, because of the low sensitivity, no ante-mortem diagnostic tests for individuals can be designed with the currently available techniques to deem animals free of infection. The interpretation of test-information will therefore rely on information available from the herd. Establishing herd-status is cumbersome with imperfect tests, but methods exists by which the probability that true prevalence is less than a specified design prevalence can be estimated. This is the probability of freedom (PFree) of infection in the population. The design prevalence is an estimate of the minimum prevalence of the infection, which the system should be able to detect in a given population. In Denmark, the prevalence of MAP infected herds has been estimated to 80-85% in 1999 (Nielsen, unpublished data). Therefore, PFree may be insufficient for classifying herds, if most herds would be classified as infected. The estimated true prevalence (TP) could be another parameter used in combination with PFree. A model by which PFree and TP could be estimated was developed for in classification of herds (Sergeant et al., 2007). This model uses age-specific sensitivities and specificities and herd demographics to provide estimates of PFree and TP. These values could subsequently be used to draw conclusions of the individual. The results of the study suggested that herds should be greater than 80 heads to provide reasonable estimates, but current test-information could be updated with previous test-information thereby increasing the precision of PFree and TP. The implementation of such a system would however be challenging, and currently it is not able to manage open herds.

DEFINITION AND USE OF COW-TYPES IN PRACTICE

Decision makers often request simple interpretation guidelines for test-results. When multiple conditions can be detected and repeated tests per animal are used, such guidelines are challenging to develop. Especially because some decision makers require a high level of detail whereas others are satisfied with a low level of detail. A simple approach can be used, namely the Bang-method, which was used for eradication of bovine

tuberculosis from Denmark (Bang, 1908; 1928). Frequent testing with an imperfect test resulted in the separation of the herd in two groups: reactors and non-reactors. The reactors should be isolated from non-reactors, and reactors which had clinically evident lesions consistent with bovine tuberculosis should be culled, resulting in three groups: 1) non-infectious, 2) apparently infected and potentially infectious, and 3) infectious and affected. Transferring this to MAP infections is possible with some modifications, and the following three groups were established: 1) non-infectious ("green" cows); 2) potentially infected and infectious ("yellow" cows); and 3) infectious and potentially affected ("red" cows). A fourth group may be considered, namely the un-tested animals ("grey" animals). The three groups can be further subdivided based on the antibody profiles, which result subsequent to repeated testing. We have defined 6 "infection groups", with antibody profiles as shown in Fig. 4. Two profiles are not shown: a) infection group 1, where only one test have been performed, and the result was negative; b) infection group 3, where 4 negative tests have followed one positive. Cows of infection group 3 are considered false-positive, but they have previously been in infection group 2 and 5. Irrespective of these further divisions into infection groups, all reactors are still considered infectious and thereby high-risk cows. However, only cows from infection groups 2 and 9 appear to have production losses (Nielsen et al., 2006). When a cow is tested positive first time (infection group 2), there is insufficient test-information to determine whether she will go to infection group 5 or 9. Therefore, it is essential that she is considered as group 9 cows until further test results are available. However, only cows of infection group 9 are recommended culled prior to next calving. A summary of the recommended decisions based on the 6 infection groups are given in Table 1. The risk groups and infection groups have currently been found to be useful and communicable to farmers and advisors, but revisions of their interpretation may be needed in light of new information.

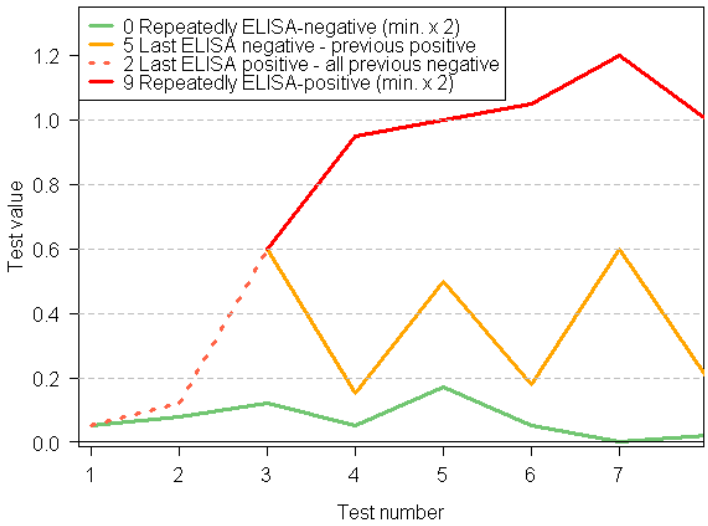


Fig. 4. Four of six antibody profiles resulting from repeated testing with a milk antibody ELISA.

Table 1. Summary of code systems and the recommended interpretation and resulting decisions of cows tested in the Danish control programme for paratuberculosis

Code system for cows			Condition			Decision
Risk group	**Colour code**	**Cow type**	**Infected**	**Infectious**	**Affected**	
Low	Green	0	?	-	-	No restrictions on use of milk and calvings can occur under regular circumstances. Also, colostrum can be used for colostrum bank. The cow cannot be deemed non-infected.
Low	Green	1	?	(-)	-	As inf. grp. 0, except colostrum should not be used for colostrum bank
High	Yellow	2	++	+++	+++	Should be considered highly infectious, but is not due for culling. More test-information is required. Could be culled if prevalence is low.
High	None	3	?	-	-	Probably a false-positive, but could be considered a potential risk as being infected.
High	Yellow	5	+	+	(-)	Moderately infectious, probably controlling infection.
High	Red	9	++	+++	+++	Should be considered highly infectious. The higher OD-value, the more infectious. Milk production is likely to be reduced, or it can be expected to occur.

REFERENCES

Bang, B., 1908. The Bang Method for the repression of tuberculosis in cattle. Commonwealth of Pennsylvania, Department of Agriculture, Bulletin no. 172, Harrisburg, Pennsylvania, USA.

Bang, B., 1928. Tuberculosis in cattle. J. Am. Vet. Med. Assoc., 72, 20-25.

Bech-Nielsen, S., Jorgensen J.B., Ahrens, P., Feld, N.C., 1992. Diagnostic accuracy of a *Mycobacterium phlei*-absorbed serum enzyme-linked immunosorbent assay for diagnosis of bovine paratuberculosis in dairy cows. J. Clin. Microbiol. 30, 613-618.

Doyle, T.M., 1953. Susceptibility to Johne's disease in relation to age. Vet. Rec., 65, 363-365.

Egan, J., Weavers, E., O'Grady, D., 1999. An evaluation of diagnostic tests for Johne's disease in cattle. Irish Vet. J., 52, 86-89.

Kudahl, A.B., Nielsen, S.S., Sørensen, J.T., 2004. Relationship between antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in milk and shape of lactation curves. Prev. Vet. Med., 62, 119,134.

Larsen, A.B., 1975. Age of cattle as related to resistance to infection with *Mycobacterium paratuberculosis*. Am. J. Vet. Res., 36, 255-257.

McKenna, S. L., Keefe, G.P., Barkema, H.W., Sockett, D.C., 2005. Evaluation of three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* using tissue and fecal culture as comparison standards. Vet. Microbiol. 110, 105-111.

McNab, W. B., Meek, A.H., Duncan, J.R., Brooks, B.W., van Dreumel, A.A., Martin, S.W., Nielsen, K.H., Sugden, E.A., Turcotte, C., 1991. An evaluation of selected screening tests for bovine paratuberculosis. Can. J. Vet. Res., 55, 252-259.

Nielsen, S.S., Ersbøll, A.K., 2006. Age at occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in naturally infected dairy cows. J. Dairy Sci., 89, 4557-4566.

Nielsen, S.S., Toft, N., 2006. Age-specific characteristics of ELISA and fecal culture for purpose specific testing for paratuberculosis. J. Dairy Sci., 89, 569-579.

Nielsen, S.S., Grønbæk, C., Agger, J.F., Houe, H., 2002. Maximum-likelihood estimation of sensitivity and specificity of ELISAs and faecal culture for diagnosis of paratuberculosis. Prev. Vet. Med., 53, 191-204.

Nielsen, S.S., Enevoldsen, C., Toft, N., 2006. Milk production losses associated with bovine paratuberculosis diagnosed from repeated testing. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, p. 619-621.

Sergeant, E.S.G, Nielsen, S.S., Toft, N., 2007. Evaluation of test-strategies for estimating probability of freedom from paratuberculosis in Danish dairy herds. Submitted to Prev. Vet. Med., March 2007.

Stabel, J. R. 2000. Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet. Microbiol. 77, 465–473.

Sweeney, R.W., Whitlock, R.H., Hamir, A.N., Rosenberger, A.E., Herr, S.A., 1992. Isolation of *Mycobacterium paratuberculosis* after oral inoculation in uninfected animals. Am. J. Vet. Res, 53, 1312-1314.

Sweeney, R. W., Whitlock, R.H., Buckley, C.L., Spencer, P.A., 1995. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. J. Vet. Diagn. Invest 7, 488-493.

Toft N, Nielsen SS, Jørgensen E, 2005. Continuous-data diagnostic tests for paratuberculosis as a multi-stage disease. J. Dairy Sci. 88: 3923-3931.

Wang, C., Turnbull B.W., Gröhn, Y.T., Nielsen, S.S., 2006. Estimating receiver operating characteristic curves with covariates when there is no perfect reference test for diagnosis of Johne's disease. J. Dairy Sci., 89, 3038-3046.

Whitlock, R.H., Wells, S.J., Sweeney, R.W., van Tiem, J., 2000. ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method. Vet. Microbiol. 77, 387-398.

Modelling paratuberculosis and effects of different control strategies

Anne Braad Kudahl[1], Søren Saxmose Nielsen[2] and Søren Østergaard[1]

[1]University of Aarhus, Faculty of Agricultural Sciences, [2]University of Copenhagen, Faculty of Life Sciences

ABSTRACT

Long term effects on paratuberculosis (PTB) prevalence and on farm-economy of following the recommendations of "Operation Paratuberculosis" (OP) were compared to alternative control strategies. In the Dub-Par project this was carried out by further development and use of the herd-simulation model PTB-Simherd. This is a dynamic, stochastic and mechanistic model which simulates the risk of infection, disease development, production effects, test results, and control actions on test result (based on choice of strategy) at cow level. Management at herd level was specified to simulate a typical Danish herd (Danish average-values, when possible). A 200 cow herd was simulated 10 years in weekly time-steps with 500 repetitions, and herd effects were evaluated. In a herd with good reproduction (heat detection rate 60%) this was repeated in 3 scenarios with initial herd prevalence of 5%, 25% and 50% respectively. I addition, an initial prevalence of 50% was simulated in a herd with a poor reproduction (heat detection rate 40%), because previous studies have found this combination to cause severe economic effects.

The simulated results indicated that:

- Recommendations of OP (special management of "yellow" and "red" cows (cows of being at high risk of being infectious) was just as effective in reducing prevalence of PTB as if all cows were specially managed to break infection routes.
- Breaking infection routes by special management was essential for the control of PTB in the herd.
- If management of infectious animals was carried out efficiently, prevalence can be reduced to 10% of the initial prevalence within 5-7 years.
- Red cows should only be culled immediately if transmission management is not optimised and reproduction is good. In any other case it is economically better to wait and cull the cow when milk yield is too low.
- The profit from controlling PTB exceeds the costs for control after 3-7 years depending on strategy.
- Compared to optimising management of all cows (without testing), joining OP is profitable whenever optimisation of management exceeds 50 DKr per calving (300 DKr when reproduction is poor)
- In herds with high prevalence of PTB and poor reproduction the economically best strategy for control of PTB is by optimising management of all cows, without testing.
- Joining Operation ParaTB without improving management is more expensive than doing nothing.
- Joining Operation ParaTB and improving management means +600 DKr pr cow/year after 10 years.

INTRODUCTION

Choosing the best control strategy against PTB in a specific herd is not easy. Farmers and advisors often ask for the effects of different strategies, both regarding economy and on the level of PTB in the herd. PTB is a slowly developing disease (Chiodini et al, 1984), and in real herds other things change so fast that the isolated effects of different strategies are impossible to estimate. In such cases simulation models are the best tool to suggest answers to such questions. A few paratuberculosis models have been developed (Collins and Morgan (1991a; 1991b; 1992), Groenendaal et al. 2002, Dorshorst et al. 2006, Kudahl et al., 2007a).

The present study is based on the PTB-Simherd model, which reflects both direct effects and indirect effects of PTB related to effects on herd dynamics and herd demographics (Kudahl, 2005). Earlier simulation studies agreed that PTB could only be controlled effectively by closing infection routes from dam to calf. However, this optimal management is rarely carried out effectively, because it implies extra hours of labour – often at inconvenient hours (when handling newborn calves). Several previous control programmes in other countries have failed for these reasons (Kennedy et al. 2001). The Danish control programme "Operation Paratuberculosis" (OP), which was initiated in February 2006, is based on the Bang method (Nielsen et al., 2006, Nielsen & Nielsen, 2007). This implies four milk-ELISA tests per year, and based on test results, cows are grouped into green (4 negative tests in a row), yellow (oscillating test results) and red cows (two last tests positive). Thereby way cows being a risk of being infectious are pointed out, and the farmer only have to optimise management of these cows instead of all cows of the herd. Time (and thereby money) are saved. But testing results in a cost along with culling of red cows before next calving, which is also recommended in OP.

The total effects of implementing these strategies in different farms are therefore far too complicated to predict with herd simulation modelling. The aim of this study was to estimate the total effects of strategies recommended in OP compared to alternative strategies (including no control) by simulations.

MATERIALS AND METHODS

The model

The PTB-Simherd model is based on Simherd, which is a dynamic, stochastic and mechanistic Monte Carlo simulation model simulating a dairy herd including young stock (Østergaard et al. 2004). The main structure of the paratuberculosis module of the Simherd model is illustrated in Fig. 1. Every cow is described by 24 parameters (e.g. milk yield, reproductive status, age, disease status), which are updated in weekly time-steps. Three of these parameters describe true PTB infection state of the animal (ovals of Fig. 1), and test results of two different diagnostic tests – in this study represented by a milk ELISA and faecal culture. Risks of infection during 4 periods of the calf-life can be specified. In case of infection the weekly risk of disease progression can be specified along with a special risk triggered by calving and other stress related events. The sensitivity and specificity of the tests can be specified according to infection stage, parity and lactation stage of the individual animal. Numerous control strategies with or without the use of testing can be specified. Production losses according to infection stage can also be specified. The model is described in detail by Kudahl et al (2007).

In the Dub-Par project, the PTB Simherd model was further developed to be able to simulate strategies of OP. A few standard input parameters were updated according to new findings: 1. risk of purchase of an infected animal was increased from 7% to 15%, 2. sensitivity of the milk ELISA of highly infectious and clinical cows was increased to 80%, and 3. Increased risk of disease progression in stressful weeks removed (calving and change in feed), and the risk of disease progression was adjusted to obtain a slower progression of infection relative to the earlier model, as a consequence of results obtained in the project (Nielsen & Ersbøll, 2006; Nielsen et al. 2006; Toft et al. 2005).

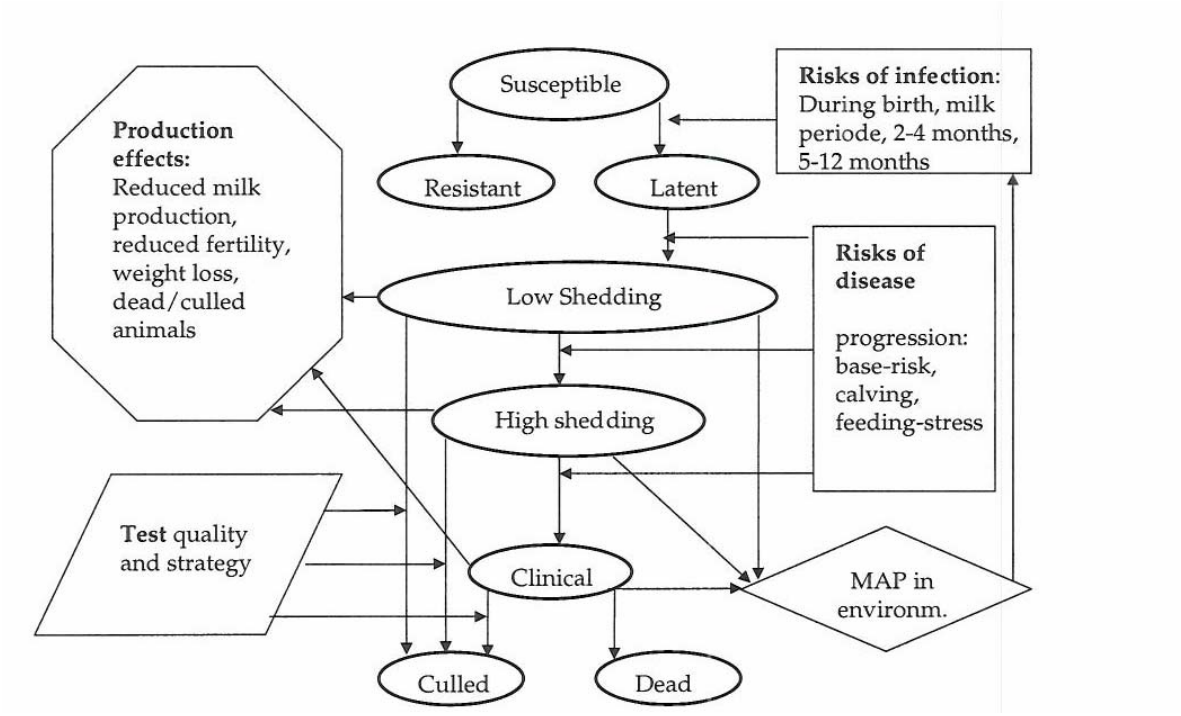


Fig. 1. Model structure of the paratuberculosis module to Simherd III. Ovals illustrate the paratuberculosis status of an animal. Boxes represent risks for infection or progression of the disease. The octahedron represents production effects. The rhomb is the environmental pool of MAP and the parallelogram illustrates the effect of test quality and strategies.

Simulated scenarios

Seven scenarios were simulated in a 200 cow dairy herd 10 years ahead with 500 repetitions. In a herd with good reproduction (heat detection rate 60%) the seven scenarios were repeated in 3 herds with initial true prevalence of 5%, 25% and 50% respectively. In addition, the seven scenarios were simulated in a herd with an initial prevalence of 50% and a poor reproduction (heat detection rate 40%), because previous studies have found this combination to have special economic effects. To compare the effects of following the recommendations of OP (with or without special management and with different culling strategies) with alternative strategies, seven scenarios were specified as follows:

1. 'No control': Default risks of infection ("average"/normal hygiene level, the calf and dam is together up to 24 hours after calving, colostrums from own dam, afterwards fed with waste-milk or bulk-tank-milk). No test and cull strategies used.

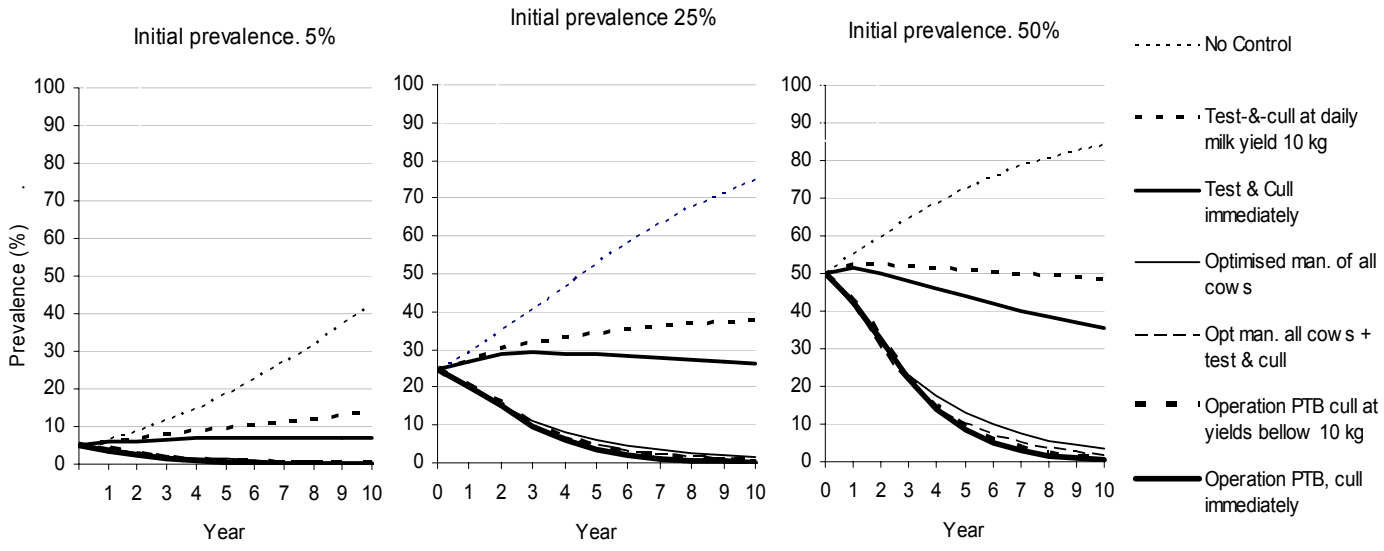
2. 'Optimised management': By breaking infection routes all risks of infection are reduced to 0.05 times default values. No test and cull strategies used.
3. 'Optimised management + culling' (Classic strategy): Like 2., but in addition all cows are tested using ELISA once a year, positive cows are confirmed by faecal culture and if confirmed, the cow is culled immediately.
4. 'Operation Paratuberculosis 1': Management of calves from all yellow and red cows is optimised to close infection routes. Insemination of red cows is stopped. Red cows culled immediately after entering the red group.
5. 'Operation Paratuberculosis 2': As strategy no. 4, but red cows are culled when daily milk yield drops bellow 10 kg, but not later than 8 weeks before next calving (due to Danish rules on slaughter of pregnant cows).
6. Test-&-cull based on testing strategies from Operation Paratuberculosis. Immediate culling of red cows (Preliminary results from Operation Paratuberculosis indicate, that the culling of red cows gives some farmers a false feeling of controlling PTB leading them to omit management improvements).
7. Like 6, but culling at a daily milk yield bellow 10 kg.

All other parameters were specified to represent a typical Danish management of a dual-purpose (milk and meat) dairy cattle herd of larger breeds with room for a maximum of 200 cows plus additional young stock. The specified replacement strategy ensured a minimum of 185 cows, and these limits define if heifers are sold or purchased. Culling effects are thus represented by the value of the slaughtered cow, the production of the replacement animal and the effect on herd dynamics. A set of Danish prices 2006 was used in the estimation of economic effects. These also include 1 hour extra labour per calving for optimising management (170 DKr / hour) (scenarios 2, 3, 4 and 5).

RESULTS

Effect on prevalence

The effect of the seven control strategies on prevalence is illustrated in Figs. 2 to 4. The ranking of the effectiveness in reducing PTB-prevalence turned out to be independent of initial prevalence. The curves demonstrate the importance of closing infection routes by optimising the management. This is done equally effective whether management is optimised for all cows (strategy 2) or only for yellow and red cows. If management is carried out optimal, prevalence can be reduced to 10% of initial prevalence within 5-7 years.

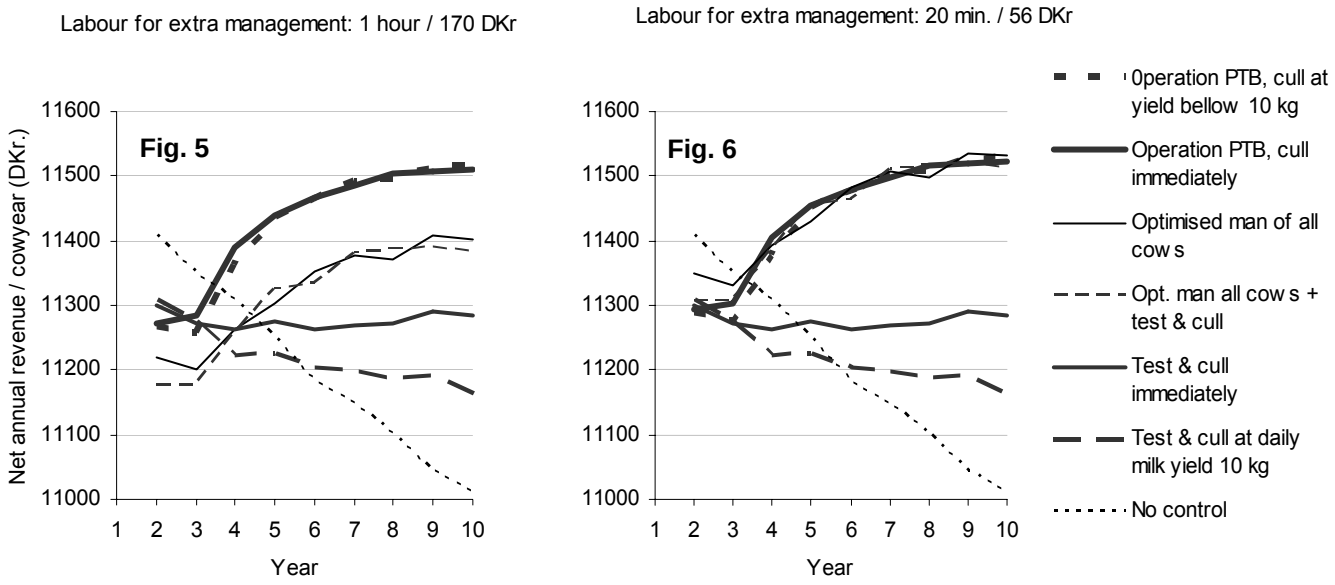


Figs. 2-4. Simulated effects on true herd-prevalence of seven different control strategies against paratuberculosis in dairy herds with an initial herd-prevalence of 5% (Fig. 2), 25% (Fig.3) and 50% (Fig.4)

Economic effects

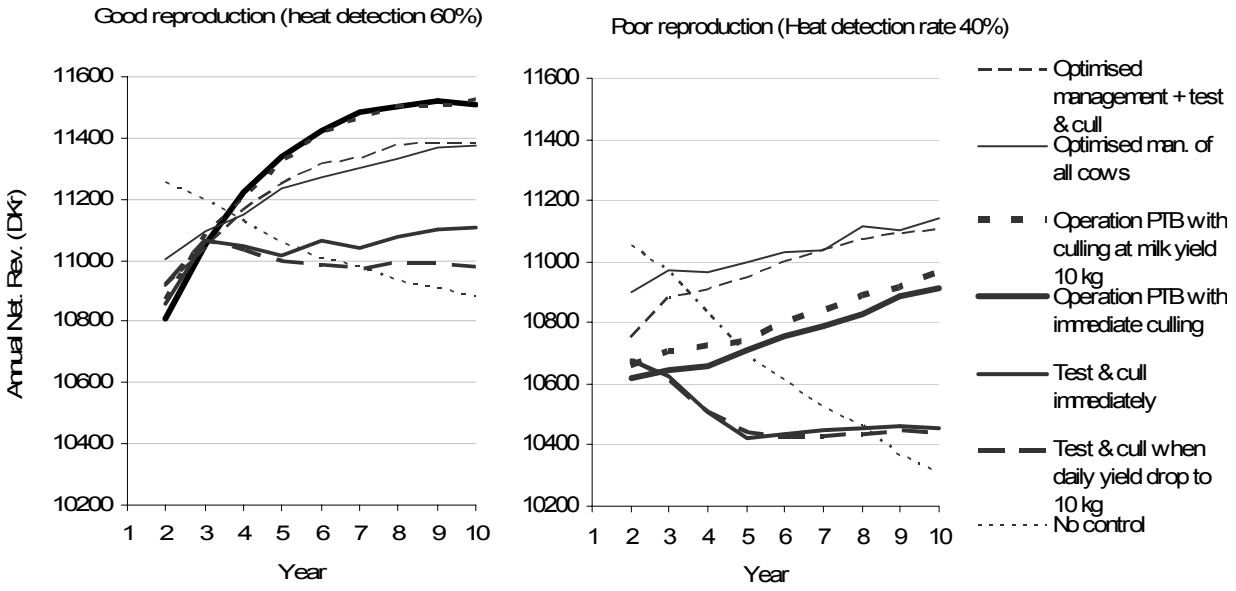
As in previous studies these simulation results point out, that if no control is performed the losses increase constantly. However, the first 3-4 years it is attempting to let things slide because costs of all strategies exceeds the profit from reducing prevalence these first years (Fig. 5). Over a period of 10 years strategies of Operation Paratuberculosis seem under these circumstances to be the economically most favourable independent on initial herd prevalence. Culling red cows immediately instead of waiting until their daily milk yield is below 10 kg has a minor positive effect, but is often of no economic importance. The second best strategy is to carry out optimal management on all cows, with or without testing and culling once a year.

These conclusions are, however, based on the assumption, that optimising management with the aim of closing infection routes takes 1 hour (170 DKr) per calf all in all (Fig. 5). Herds are however different and in some herds it might be easier to change management. Fig. 6 show the economic effects in a herd where the extra management only takes 20 minutes per calf. In this case optimising management of all cows without testing is most profitable the first 3 years (due to no expenses for tests and cullings). After this point that strategy (with or without using tests once a year) have the same economic effects as joining OP. This means, that if extra labour to optimise management is estimated to take less than 20 min. per calf (or cost less than about 50 DKr) it is economically favourable to optimise management of all cows instead of joining OP, which implies the costs for tests and culling.
The way the model estimate the economic results of the first year is misleading, and they have therefore been excluded from the figures.



Figs. 5-6. Economic effects of seven different control strategies in a herd with 25 % initial true herd prevalence and a good reproduction (heat detection 60%) calculated as net annual revenue per cow per year. In Fig. 5 extra labour for closing transmission routes by optimising management is assumed to take 1 hour/ cost 170 DKr for each calf. In Fig. 6. this extra labour is assumed to take 20 minutes / cost 56 DKr.

The simulated economic effects of reproduction quality in herds with high prevalence are illustrated in Figs. 7 - 8. The general economic effect of poor reproduction (heat detection rate 40%) compared to good reproduction means a loss of 200 – 800 DKr per cow per year depending on control strategy. The poor reproduction means that strategies including testing and culling have a much more server effect on the economy, because of lacking replacement animals. This makes strategies of optimising management for all cows (without or only with a few cullings) economically much more favourable than OP strategies, which imply culling of all red cows. Poor reproduction also increases the importance of time of culling in OP. It is profitable to wait and cull the cows until their milk yield is too low. This is, however, not the case if the tests are only used for culling and transmission routes are not closed. On these farms the advantage of keeping the cows longer is counterbalanced by the disadvantages of letting these high-shedders continue to spread the infection. In fig. 7.and 8. extra labour for optimising management is set to 1 hour per calf. The threshold where joining OP becomes profitable compared to optimising management without tests is now increased to 300 DKr per calf (not shown). These means that on farms with this special combination of high prevalence and poor reproduction focus on closing transmission routes is even more important because culling of cows is much more expensive, than when reproduction is good.



Figs. 7-8. Simulated economic effects of different control strategies against PTB in farms with high initial prevalence (50%) and with good (**Fig. 7**) and poor (**Fig. 8**) reproduction (heat detection rates of 60% vs. 40%). Extra labour for optimising management to close infection routes is set to 1 hour per calf.

DISCUSSION, CONCLUSIONS AND PERSPECTIVES

Operation Paratuberculosis initiated in February 2006 and there are still only a few results, and analysis of the effects of using the Bang–method for controlling PTB has never been performed before. Validation of the simulations is thereby only possible by face-validation: Do the results seem possible and explainable?

Previous studies have though confirmed the importance of closing transmission routes and found poor effects of controlling PTB only by testing and culling (Groenendaal et al., 2002, Dorshorst et al., 2006; Kudahl et al, 2007a, b).

In summary the simulations indicate that:

- Following recommendations of OP is just as effective in reducing prevalence of PTB as if all cows were specially managed to break infection routes.
- Breaking infection routes by special management is essential for the control of PTB in the herd.
- If management is carried out optimal, prevalence can be reduced to 10% of initial prevalence within 5-7 years.
- Red cows should only be culled immediately if management is not optimal and reproduction is good. In any other case it is economically better to wait and cull the cow when milk yield is too low.
- The profit from controlling PTB exceeds the costs for control after 3-7 years depending on strategy.
- Compared to optimising management of all cows (using no tests), joining OP is profitable whenever this special management exceeds 50 DKr per calving in herd with good reproduction (heat detection success of 60%) and 300 DKr when reproduction is poor
- In herds with high prevalence and poor reproduction the economically best way to control PTB is by optimising management of all cows and use no tests – if manpower for extra labour is available.
- Joining Operation ParaTB **without** improving management is more expensive than doing nothing.
- Joining Operation PTB **and** improving management means up to +600 DKr pr cow/year after 10 years.

The present study thus underlined that the profitability of joining OP is very sensitive to the herd-specific reproduction quality and necessary amount of extra labour related to optimising management.

Although these simulations indicate, that under many conditions it is more profitable to use no tests and manage all cows optimally instead of joining OP, experiences from several countries show that this strategy is only practicable for a few farmers (Kennedy et al. 2001, Groenendaal, 2003). The burden of this labour is too heavy and often at inconvenient hours. Sources for the extra hours of labour are often not available due to scarcity of manpower. In these cases the strategies of Operation Paratuberculosis probably show a more manageable way of controlling PTB in an effective way. Based on the large economic effects of cullings on farms with a poor reproduction the importance of culling red cows on these farms could be

reconsidered and analysed by further simulations. However, there are still important ethical and environmental (and maybe biosecurity) reasons to cull the most infectious and diseased cows rather quickly.

Another aspect which is not included in the simulation model is that an important part of Operation PTB is the communicative learning and motivating process of working with the manual. The economic value of this process is not included, but the educating and motivating effect on the farmer is crucial to the success of the control programme.

REFERENCES:

Chiodini, R.J., van Kruiningen, H.J., Merkal, R. S. 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet. 74, 218-262.

Collins, M.T. and Morgan, I.R. 1991a. Epidemiological model of paratuberculosis in dairy cattle. Prev. Vet. Med. 11: 131-146.

Collins, M. T. and Morgan, I.R. 1991b. Economic decision analysis model of a paratuberculosis test-and-cull program. J. A. Vet. Med. Assoc.199: 12, 1724-1729.

Collins, M. T. and Morgan, I.R.1992. Simulation model of paratuberculosis control in a dairy herd. Prev. Vet. Med., 14: 21-32.

Dorshorst, N.C., Collins, M. T. Lombard, J. E. 2006. Decision analysis model for paratuberculosis control in commercial dairy herds. Prev. Vet. Med. 75, 92-122.

Groenendaal, H., Nielen, M., Jalvingh, A.W., Horst, S.H., Galligan, D.T., Hesselink, J.W. 2002. A Simulation of Johne's disease control. Prev. Vet. Med. 54, 225-245.

Groenendaal, H., Nielen, M., Hesselink, J.W. 2003. Development of the Dutch Johne's disease control program supported by a simulation model. Prev. Vet. Med. 60, 69-90

Kennedy, D., Holmström, A., Plym Forshell, K., Vindel, E., Suarez Fernandez, G. 2001. On-farm management of Paratuberculosis (Johne's disease) in Dairy Herds. Bulletin of the International Dairy federation 362, 18-31.

Kudahl, A.B., 2005. Economic Consequences of Paratuberculosis Control in Dairy Herds. PhD thesis. DIAS report no. 63, June 2005.

Kudahl, A.B., Sørensen, J.T., Nielsen, S.S., Østergaard, S., 2007a. Simulated economic effects of improving the sensitivity of a diagnostic test in paratuberculosis control. Prev. Vet Med., 78, 118-129.

Kudahl, A.B., Østergaard, S., Sørensen, J.T., Nielsen, S.S., 2007b. A stochastic model simulating paratuberculosis in a dairy herd. Prev. Vet. Med. 78, 97-117.

Toft, N., Nielsen, S.S., Jørgensen, E., 2005. Continuous-data diagnostic tests for paratuberculosis as a multi-stage disease. J. Dairy Sci. 88: 3923-3931.

Nielsen, S.S., Ersbøll, A.K., 2006. Age at occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in naturally infected dairy cows. J. Dairy Sci. 89, 4557-4566.

Nielsen, S.S., Toft, N., 2006. Age-specific characteristics of ELISA and fecal culture for purpose specific testing for paratuberculosis. J. Dairy Sci. 89: 569-579.

Nielsen, S.S., Jepsen, Ø.R., Aagaard, C. 2006. Control programme for paratuberculosis in Denmark. Proccedings of the 1st ParaTB Forum. October 19. 2006, Shanghai, China, p. 25-30.

Nielsen, S.S., Nielsen L.R., 2007. Indsats mod Paratuberkulose og *Salmonella* Dublin. Rådgivermanual til intervention mod paratuberkulose og *Salmonella* Dublin. 5nd Edition. (In Danish, English title: Manual for advisors: Intervention against paratuberculosis and *Salmonella* Dublin). University of Copenhagen/ Danish Cattle Federation, 42 pp.

Østergaard, S., Sørensen, J.T. Enevoldsen, C. 2004. Simherd III: User's Manual. PC Programmes for simulation and analysis of production and health in the dairy herd. Internal report no 209. 2004. Danish Institute of Agricultural sciences.

Intervention against *Salmonella* Dublin and Paratuberculosis

Søren Saxmose Nielsen and Liza Rosenbaum Nielsen

University of Copenhagen, Faculty of Life Sciences, Dept. of Large Animal Sciences

ABSTRACT

Intervention against paratuberculosis and *Salmonella* Dublin should focus on management of transmission of the causative bacteria. Herd-specific approaches are usually required to manage these infections, which are often latent in a number of animals within a given herd. Latently infected animals as well as infectious and affected animals should all be managed to avoid transmission of the infections, but the specific actions taken may differ from one group of animals to another, and from herd to herd.

Diagnostic test results are not required to control or eradicate the infections, but diagnostic testing can be used as a tool for management of the infections, as well as they can be used to monitor the successes or failures and for motivation in general.

We describe the principles of the approaches used to manage paratuberculosis and *S.* Dublin, as well as results and practical experiences from 16 herds, which have intervened against one or both infections in a 3-year period. We found that: a) it is feasible to implement the most important changes of management procedures to reduce transmission in most herds, but the ideal practice is not always implemented. Instead, alternative measures are included; b) diagnostic test-results on animal level can be of assistance for management of the infection, particularly for paratuberculosis and to a smaller extent for *S*. Dublin; c) diagnostic tests can be used for monitoring successful implementation of changes of management for *S.* Dublin but have yet to be implemented for paratuberculosis; d) tools for information and communication need to cover a wide spectrum for the messages to reach all farmers, but it is feasible to manage paratuberculosis and *S.* Dublin in most herds, often at limited costs.

INTRODUCTION

Management of herds infected with *Salmonella* (*S.*) Dublin and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) usually requires breaking the major routes of transmission. Test-and-cull strategies are not cost-effective if prevention of within-herd transmission is in place. Scientific evidence of the major risk factors for within-herd transmission is limited. General knowledge of transmission is mainly based on empirical knowledge. A major reason for lack of such evidence is probably the latent stages of infection, which both infections can result in and the lack of sensitivity and sometimes specificity of available diagnostic tests. Another reason is that herd systems to a great extent are different from herd to herd, and their inclusion in scientific studies poses major challenges.

The current empirical knowledge suggest that: younger animals are more susceptible to both infections than older; a number of animals carry a latent infection, which may evolve into bacterial shedding, but this need not happen at all; some animals may develop into super-shedders for shorter or longer periods of their life-time. The situation where most infections are considered to take place, common for the two infections is from infectious animals to susceptible newborn calves in the calving area. *S*. Dublin can be transmitted from infectious animals to most other age-groups at any point in time, suggesting that strict grouped housing is recommendable and preweaned calves should be housed in clean environments one or two together with solid walls separating them from their neighbours to avoid faecal contamination. MAP infections via milk and in some case in utero should also be considered. For both infections, it should be considered that any manure originating from other animal groups should not come into contact with susceptible animals.

Reduction in transmission of *S*. Dublin and MAP can be achieved partly via changes of management and housing of animals, partly via diagnostic testing and timely removal of infectious animals. Management of the infections requires long-term and daily efforts, and the required action plans will often differ from one herd to another. Therefore, we have deemed it important that herd-managers should have a thorough understanding of the infections, in order to manage them as cost-effectively as possible throughout a control programme. Consequently, communication is a major challenge in control programmes. This includes communication between central decision makers, farmers and local advisor. The communication can be assisted by tools, which convey information of infection status of herds and individuals, obtained via diagnostic test information. Both national programmes and local herd intervention strategies should therefore contain three components: a) reduction of transmission via changes in management; b) culling of infectious animals; and c) communication.

COMMUNICATION

Communication is considered a main component in control programmes against infections such as paratuberculosis (Kennedy and Padula, 2006) and *S.* Dublin. Education of farmers, advisors and authorities is a necessary component in any programme on infectious diseases (Yekutiel, 1980). It is necessary that farmers are quite knowledgeable about *S*. Dublin and paratuberculosis, if they are to control these infections,

particularly because test-and-cull schemes are not readily available and useful, and because the efforts will usually have to continue for several years. Therefore, a manual for advisors (Nielsen and Nielsen, 2007) was created inspired by an American manual for paratuberculosis control (Rossiter et al., 1999). The Danish manual was adapted for Danish conditions and revised according to relative risks deemed important in the Danish production systems. It was adjusted according to feedback from users throughout the project period. Also, a background section was included to provide the most important knowledge about the two infections. The manual is recommended to be used by herd health advisors to: a) teach farmers about the infections and their transmission routes and patterns; b) to establish herd-specific goals regarding the infections and short and long-term production goals; c) to make a herd-specific risk assessment; d) to make an action plan; and e) to provide basic schemes for use and interpretation of test-results (Nielsen and Nielsen, 2005).

Herd health advisors (practising veterinarians and animal scientists) can obtain the manual from the internet. A total of 5 courses about practical handling of paratuberculosis and *S*. Dublin including how to use the manual have been held in the period 2004 to 2006. Besides the manual, a number of professional papers have been written in farmers' magazines, a separate homepage for paratuberculosis (http://www.paratuberkulose.dk) have been created and a DVD about paratuberculosis was sent to all farmers in November 2006. ERFA-meetings (experience meetings) with the participation of farmers and their herd advisors are also taking place to some extent, and may be a very productive method for conveying information about the two infections.

TEST INFORMATION FOR *S*. DUBLIN

In October 2002, a national surveillance programme was initiated to classify all cattle herds in Denmark into three infection levels thereby providing farmers with a tool to protect their herds from risks of introducing infected animals to their herds upon purchase and other contacts between herds. In dairy herds the classification is based on testing of antibodies (ELISA) in bulk tank milk samples collected routinely through the milk quality control scheme. In non-dairy herds the classification is based on blood samples collected at slaughter (Nielsen et al., 2006). The classification levels are publicly available on the internet (www.glr-chr.dk) and ELISA results from the surveillance programme are available to farmers and their advisors through the herd management software provided by the Danish Cattle Federation (Dyreregistrering®) and can be used to give an reasonably good idea about the prevalence of infection in the herd (Nielsen and Ersbøll, 2005). Individual *Salmonella*-ELISAs have been developed to test both young stock with blood samples and lactating cows with blood or yield control milk samples. The ELISAs have been evaluated for sensitivity and specificity of *S*. Dublin infections and use in relation to evaluation of transmission of bacteria and risk of shedding in the individual (Nielsen, 2003; Nielsen and Ersbøll, 2004; Nielsen et al., 2004; Nielsen et al., 2007). Bacteriological culture of faecal samples has also been evaluated as a tool to detecting shedding animals. The sensitivity has been found to be poor due to intermittent shedding, low concentrations of bacteria in the samples and other factors related to the origin of the faecal material and the strain of bacteria (Nielsen et al., 2004, Baggesen et al, 2007). It may still be used to confirm clinical outbreaks.

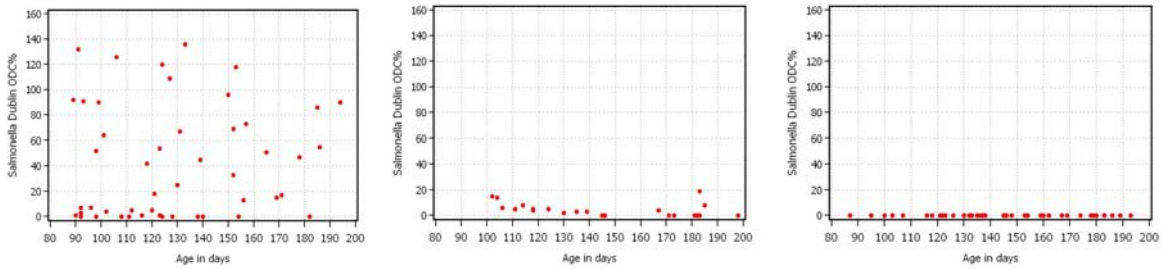


Fig. 1. Antibody measurements (*Salmonella* Dublin ODC%) in serum samples from calves aged approximately 3-6 months in a dairy herd before (left graph), six months after (middle graph) and one year after (right graph) effective intervention was initiated.

In Denmark today, it is recommended that bulk tank milk ELISA results are used to evaluate the risk of infection in the herd. Level 2 herds in the national surveillance programme have a high risk of being infected and usually there will be transmission going on among the calves and young stock when the bulk tank milk is high, stable above 40 ODC% or recently increased markedly. If this is the situation in a herd that wants to eradicate the infection, it is recommended that individual ELISA measurements are only used to evaluate if the intervention tasks performed in the herd are working by testing calves between 3-6 months of age. Fig. 1 illustrates how the ELISA-results differ in a herd with active and no transmission of infection among calves before and after intervention has been initiated.

After the intervention has been ongoing and has been successful for about one year, it can be supplemented with detection of high risk animals of in-calf heifers and adult cows as suggested in Nielsen et al. (2007). Culling is not necessarily the best way to handle all the high risk animals. In particular multiple

parity cows have fairly low risk of shedding even when they have persistently high antibody measurements. But care must be taken that they are not allowed to spread the infection to their calves around calving.

TEST INFORMATION FOR PARATUBERCULOSIS

Ideally, control of MAP can be achieved without use of diagnostic tests (Kudahl et al., 2007). However, diagnostic test results can be neat to have for daily management of the infection, for success monitoring and for motivational purposes. In the Danish tuberculosis eradication programme, the Bang method was used (Bang, 1908; 1928). Briefly, the Bang method is based on frequent testing of all cattle in the herd by use of an imperfect test. Reactors should be isolated from non-reactors, and highly infectious animals should be culled as soon as possible. For paratuberculosis, frequent testing would only be practically feasible and cost-effective if done via testing of milk samples obtained from the milk recording scheme. Although the antibody ELISA used is imperfect, it is possible to detect most infectious animals in due time in order to prevent transmission to susceptible animals (Nielsen, 2007). Also, the test-information can be used to estimate the production losses, which is apparently caused by the MAP-infection (Nielsen, 2007), and therefore be used as a motivational tool. A tool for monitoring of the infection within the herd has yet to be established.
The results from the diagnostic testing are presented as follows:

1) Management lists, where cows are divided into High-risk (Red and Yellow), Low-risk (Green) and untested animals (Fig. 2). The lists are used for management around calving and milk feeding. “Red” and “Yellow” cows are high-lighted with red and yellow colours, respectively.
2) List of last six antibody recordings for each cow, divided into 6 “infection-groups”. These lists provide an overview of the antibody profile for each cow;
3) Culling-list, where cows recommended for culling are listed;
4) A crude estimate of the production losses, which are calculated to be caused by the MAP infection in the herd (Fig. 3).

Dansk Kvæg		ParaTB Milk feeding list
Herd with 230 cows	Herd ID xxxxx Test date: 15.02.06	Printed 23.08.06 15.38 Page 1 Den Kgl. Veterinær- og Landbohø 35 28 20 79 135

Based on milk antibody test i Herd xxxxx tested 15.02.06

Risk = High: milk should not be used for feeding of heifer calves; High hygiene around calving

Animal ID	ELISA (15.02.06)	Previous (04.01.06)	Clv.no.	Exp. calving	Milk yield drop	Inf.-group
-00929	0.9	0.1	6		Likely	2#
-00940	0.3	0.7	4	09.10.06	Very likely	9*
-00941	1.0	1.2	5	28.09.06	Very likely	9*
-00958	0.5	1.0	5	10.03.07	Very likely	9*
-00965	0.7	0.0	5		Likely	2#
-00982	0.8	0.8	4	18.09.06	Very likely	9*
-00984	0.6	0.6	3	23.11.06	Very likely	9*

Fig. 2. Extract of a management list used for management of calving and milk feeding, based on test results in a herd regularly tested with a milk antibody ELISA. Only a part of the High-Risk cows are shown, and none of the Low-Risk cows are shown.

RESULTS FROM 16 INTERVENTION HERDS

Implementation of action plans to manage S. Dublin and paratuberculosis

Intervention against *S*. Dublin and paratuberculosis was initiated in 19 herds in late 2003 /early 2004. In these herds, the local veterinarians were asked to provide advisory services regarding the two infections. Thirteen of the herds were in *S*. Dublin Level 2 at the beginning of the project. MAP has been isolated from all but one herd at least once during the project period. In the herd where MAP has not been isolated, the sero-prevalence suggests that MAP is present.

Each herd was tested: i) 4 times annually for MAP and *S*. Dublin antibodies in milk among all lactating cows; ii) twice annually for *S*. Dublin in serum among young stock aged 3 months and higher (until 1st calving); iii) once annually for MAP in faeces from all cows. The costs for these samples were paid by the project, whereas costs related to advisory services were paid by the farmer at regular costs. The test results were communicated for farmers to assist in management of infected and infectious cows. The presentation of the results in management lists changed over the project period until a format was found by which the desired information could be conveyed to the farmers and advisors. The management lists included systems

in which cattle were group into a high-risk group and a low-risk group. The high-risk group was further divided into “Red” and “Yellow” cows, where the former should be considered very infectious whereas the latter should be considered infected and potentially infectious. The recommendations to farmers were that transmission of *S*. Dublin or MAP from high-risk animals should be eliminated via appropriate measures. These appropriate measures should be identified via the risk assessments in the manual for advisors. Some of the high-risk animals should be culled. Initially, these were identified manually by the project leaders (Nielsen LR and Nielsen SS) whereas this process was partly made automatic in the course of the project.

		1st Parity cows	2nd Parity cows	> 2nd Parity cows	Total
Avg. kg. ECM		7581	8679	9407	
No. of cows	**Inf.-grp**				
	0	74	63	88	225
	2	1	3	3	7
	9	0	2	14	16
Annual production loss		910	4773	17592	23275
	Inf.-grp				
Percent cows	**2+9**	1	7	16	9

Fig. 3. Estimated milk production losses in the herd is estimated based on the potential production among cows of infection group 0 and the no. of cows in infection groups 2 and 9. The losses are rough estimates and are based on a percent-wise reduction compared to what is expected in an age-group. In this herd with approximately 300 cows, 248 were classified and of these, 9% were estimated to have a production loss corresponding to a total of 23 tonnes energy corrected milk (ECM).

Summary of interviews

Success of implementation of the required intervention measures was evaluated through an interview of the herd-managers in the project in October – November 2006. Three of the herds had ceased production, two of which were S. Dublin Level 2. Therefore, only 16 herds were visited. The objective of the interviews was to assess whether the necessary changes of routines and housing had been implemented in the herds. For *S.* Dublin, this could partly be assessed via the use of test-results from bulk tank milk samples and serum-antibody results of young stock as shown in Fig.1. For MAP, the results of testing could mostly be used for management of high-risk cows and culling of the most infectious animals. It was investigated to what extend the herd-managers had used these results for management of the infection. The farmers were asked to explain how they had made the necessary changes, and the validity of their statements was assessed through subjective judgment of plausibility, i.e. could the changes be made as stated or had it been demonstrated that it was feasible. Management related to the following parameters was subject to the interviews: Management related to milk feeding; management related to procedures around calving; timely culling of infected and infectious animals.

The interviews indicated that:

a) Most herds could implement procedures, through which feeding of milk (colostrum, waste milk and milk from cows with high somatic cell counts / containing antibiotics) could be avoided from high-risk cows. In one herd, the farmer had chosen to use bulk tank milk for feeding of calves, but he was aware of the risk associated with this strategy;
b) Fast removal of calves born by high-risk cows was generally practised, i.e. if a high-risk dam was giving birth, the calving area was visited more frequently than for low-risk animals. This was practised more or less systematically in all herd;
c) Cleaning of calving facilities between calvings was generally not practised, as it was deemed to be too time-consuming in most systems. Four herds did practice cleaning between calvings. In these herds, the calving areas were constructed so that it was easy to manage and not really time-consuming.
d) When cleaning of calving facilities did not take place, high amounts of straw on top of the current bedding in the calving areas usually was used as an alternative. This should reduce the risk of transmission according to one risk factor study (Nielsen and Toft, 2007).
e) Separate calving facilities for high-risk and low-risk cattle can be established in most herds, but this is often not done;
f) Culling of test-positive cows was done semi-aggressive in the beginning of the project (more aggressive than was recommended), but later more moderate culling was performed in most herds, according to the recommendations. Some herd-managers were reluctant to cull some animals, which were evaluated to be very infectious. These animals were usually cows or heifers of high genetic value.

The herd-managers were also asked to state where they had gained the knowledge and motivation, and when they had made different changes. There were major differences in where the information had been obtained: Some had read the manuals from one end to the other. One farmer used the risk assessments as a teaching-tool for new employees. Some had most information from professional publications in farmers' magazines or from various farmers' meetings. Some had their knowledge from discussions with their herd veterinarian. Most of them had experienced the highest gain of information from an experience meeting held midway in the project. Here farmers could discuss with fellow farmers what they had experienced to work and not work. These meetings were held in groups of 8 to 9 farmers plus their local advisors in June 2005. Many of the farmers stated that they had only really implemented the management changes after this meeting, where they had also been motivated by the apparent major production losses, which were demonstrated to occur due to MAP infections (Nielsen et al., 2006a). There seemed to be a change in attitude towards MAP infections throughout the course of the project: 16 of the farmers stated that it was important to focus on paratuberculosis in the end of the project, whereas 5 to 10 farmers were reluctant to participate in the project initially because of fear of their herd testing positive.

Progress in intervention against *S.* Dublin turned out to be reasonably easy to evaluate by the use of biannual blood sampling of young stock in the herds as illustrated in Fig.1. These graphs which in the project were provided for all animals and not just 3-6 months old calves were highly motivating for most farmers when they showed that the intervention that had been performed over the last 6-7 months had been working. If intervention in the calf barn is effective, all antibody measurements of calves between 3-6 months should be very close to 0 ODC% showing that the animals have not been exposed to *S*. Dublin bacteria. After 1-2 years depending on starting prevalence of infection the effect of the intervention also became evident on the bulk tank milk ELISA measurements. Nine out of 13 herds that started in Level 2 reached levels of antibody measurements required to move to Level 1 within the project period, two ceased production and two were clearly making improvements in the young stock, but did not reach Level 1 before the project stopped.

PLANS AND PERSPECTIVES

S. Dublin eradication campaign

The Danish Cattle Federation (DCF) and the Food and Veterinary Administration have agreed to the following plan for eradication of S. Dublin from the Danish cattle population before end of 2014:

Phase 1: Voluntary intervention in cattle herds with technical support from DCF and local advisors (2007-2009) Farmers will be encouraged to use the principles developed in this project.
Phase 2: Sanctions may be imposed on infected herds by differential milk/meat pricing (2010-2012)
Phase 3: Regulatory restrictions may be imposed to control infection (2013-2014)

Operation Paratuberculosis

In February 2006, DCF launched a voluntary control programme, called Operation Paratuberculosis (Nielsen et al., 2006b). In February 2007, 1051 (20%) of the 5118 Danish dairy herds had signed up for the programme. The aims of the programme are: 1) to reduce the overall prevalence of paratuberculosis in Denmark; 2) to provide tools to farmers that wish to control paratuberculosis.

The principles in the programme are those described as the Bang method (Bang, 1908; 1928): Frequent testing is conducted with a sensitive but not necessarily very specific test. Farmers are currently required to get four annual herd tests, including all lactating animals. Test-information is presented as stated in the section "Test-information for Paratuberculosis". Farmers are encouraged to: a) reduce transmission from "Red" and "Yellow" cows; and b) cull red cows prior to next calving if possible. Also, they are advised to seek assistance from their herd health advisor to establish goals regarding the infection, perform a risk assessment, establish an action plan to reduce transmission and describe how they will use test-results

The future plans for the paratuberculosis programme have not been elaborated further, except that the efforts of farmers need to be evaluated. There is currently no surveillance component in the programme, and a herd status is not given to each herd. Given there is a political request for a surveillance or herd certification, it is likely that it will include the estimation of the probability of freedom of a given herd combined with a true (calculated) prevalence. Both are intended to operate on continuous scales, without classifying herds into infected and non-infected. The system is described in further detail in Sergeant et al. (2007).

REFERENCES

Baggesen, D.L., Nielsen, L.R., Sørensen, G., Bodker, R. and Ersbøll, A.K., 2007. Growth inhibitory factors in bovine faeces impairs detection of *Salmonella* Dublin by conventional culture procedure. J Appl. Microbiol. (Available online: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2672.2007.03292.x)

Bang, B., 1908. The Bang Method for the repression of tuberculosis in cattle. Commonwealth of Pennsylvania, Department of Agriculture, Bulletin no. 172, Harrisburg, Pennsylvania, USA.

Bang, B., 1928. Tuberculosis in cattle. J. Am. Vet. Med. Assoc., 72, 20-25.

Kennedy, D, Padula, A.M., 2006. Review of International Communication and Training Programs for Johne's Disease (paratuberculosis) in Dairy Cattle. Proceedings of the 1st ParaTB Forum, 19 October 2006, Shanghai, China, p. 43-48.

Kudahl, A.B., Østergaard, S., Sørensen, J.T., Nielsen, S.S., 2007. A stochastic model simulating paratuberculosis in a dairy herd. Preventive Veterinary Medicine, 78, 97-117.

Nielsen, L.R., 2003. *Salmonella* Dublin in dairy cattle: Use of diagnostic tests for investigation of risk factors and infection dynamics. PhD Thesis. The Royal Veterinary and Agricultural University.

Nielsen, L.R., Baggesen, D.L., Ersbøll, A.K., 2007. Challenging the traditional methods for detection of *Salmonella* Dublin carrier animals. Proceedings DubPar-seminar, Technical University of Denmark, Copenhagen, 22 March 2007, pp. 12-17.

Nielsen, L.R., Ersbøll, A.K., 2004. Age stratified validation of an indirect *Salmonella* Dublin serum ELISA for individual diagnosis in cattle. J. Vet. Diagn. Invest. 16, 205-211.

Nielsen, L.R., Ersbøll, A.K., 2005. Factors associated with variation in bulk-tank-milk *Salmonella* Dublin ELISA ODC% in dairy herds. Prev. Vet. Med. 68, 165-179.

Nielsen, L.R., Rattenborg, E. and Nielsen, J., 2006. Development of the National Surveillance Programme for *Salmonella* Dublin in Danish cattle. *In*: Proceedings of the 11th International Symposium for Veterinary Epidemiology and Economics (ISVEE), Cairns, Australia, August 2006, p. 870.

Nielsen, L.R., Toft, N., Ersbøll, A.K., 2004. Evaluation of an indirect serum ELISA and a bacteriological faecal culture test for diagnosis of *Salmonella* serotype Dublin in cattle using latent class models. J Appl. Microbiol. 96, 311-319.

Nielsen, L.R., Nielsen, S.S., 2005. Risk assessment and management strategies to control *Salmonella* Dublin and paratuberculosis in dairy herds. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, no. 510.

Nielsen, S.S., 2007. Interpretation of diagnostic test-information for paratuberculosis control and surveillance Proceedings DubPar-seminar, Technical University Denmark, Copenhagen, 22 March 2007, p. 27-32.

Nielsen, S.S., Nielsen, L.R., 2007. Indsats mod Paratuberkulose og *Salmonella* Dublin, 5th ed. (2 parts). (http://www.lr.dk/kvaeg/diverse/netudgave.htm).

Nielsen, S.S., Toft, N., 2007. Assessment of management-related risk factors for paratuberculosis in Danish dairy herds using Bayesian mixture models. Submitted to Preventive Veterinary Medicine, Aug. 2006.

Nielsen, S.S., Enevoldsen, C., Toft, N., 2006a. Milk production losses associated with bovine paratuberculosis diagnosed from repeated testing. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, p. 619-621.

Nielsen, S.S., Jepsen, Ø.R., Aagaard, K., 2006b. Control programme for paratuberculosis in Denmark. Proceedings of the 1st ParaTB Forum, Shanghai, China, Oct. 19, 2006, p. 21-26.

Rossiter, C.A., Hutchinson, L.J., Hansen D., Whitlock R.H., 1999. Johne's disease prevention/ control plan for beef herds. Manual for Veterinarians. The Bovine Practitioner, 33, 194-1–194-22.

Sergeant, E.S.G, Nielsen, S.S., Toft, N., 2007. Evaluation of test-strategies for estimating probability of freedom from paratuberculosis in Danish dairy herds. Submitted to Prev. Vet. Med., March 2007.

Yekutiel, P., 1980. Eradication of infectious diseases: A critical study. In: Contribution to Epidemiology and Biostatistics, vol. 2, Karger, Basel, Switzerland, 164 pp.

Publications resulting from the DubPar project

PUBLICATIONS IN INTERNATIONALLY PEER-REVIEWED PERIODICALS

Baggesen DL, Nielsen LR, Sørensen G, Bødker R, Ersbøll AK, 2007. Growth inhibitory factors in bovine faeces impairs detection of *Salmonella* Dublin by conventional culture procedures. Journal of Applied Microbiology. Online March 2007: doi:10.1111/j.1365-2672.2007.03292.x

Hansen KR, Nielsen LR, Lind P, 2006. Use of IgG avidity ELISA to differentiate acute from persistent infection with *Salmonella* Dublin in cattle. Journal of Applied Microbiology, 100, 144-152.

Kudahl AB, Nielsen SS, Sørensen JT, 2004. Relationship between antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in milk and shape of lactation curves. Preventive Veterinary Medicine, 62, 119-134.

Kudahl AB, Østergaard S, Sørensen JT, Nielsen SS, 2007. A stochastic model simulating paratuberculosis in a dairy herd. Preventive Veterinary Medicine, 78, 97-117.

Kudahl AB, Sørensen JT, Nielsen SS, Østergaard S, 2007. Simulated economic effects of improving the sensitivity of a diagnostic test in paratuberculosis control. Preventive Veterinary Medicine, 78, 118-129.

Mortensen H, Nielsen SS, Berg P, 2004 Genetic variation and heritability of the antibody response to *Mycobacterium avium* subsp. *paratuberculosis*. Journal of Dairy Science, 87, 2108-2113.

Nielsen LR, van den Borne B, van Schaik G, 2007. *Salmonella* Dublin infection in young dairy calves: Transmission parameters estimated from field data and an SIR-model. Preventive Veterinary Medicine 79, 46–58.

Nielsen LR, Schukken YH, Gröhn YT, Ersbøll AK, 2004. *Salmonella* Dublin infection in dairy cattle: Risk factors for becoming a carrier. Preventive Veterinary Medicine, 65, 47-62.

Nielsen SS, Ersbøll AK, 2006. Age at occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in naturally infected dairy cows. Journal of Dairy Science, 89, 4557-4566.

Nielsen SS, Toft N, 2006. Age-specific characteristics of ELISA and fecal culture for purpose specific testing for paratuberculosis. Journal of Dairy Science, 89: 569-579.

Skovgaard KI, Grell SN, Heegaard PM, Jungersen G, Pudrith CB, Coussens PM, 2006. Differential expression of genes encoding CD30L and P-selectin in cattle with Johne's disease: progress toward a diagnostic gene expression signature. Veterinary Immunology and Immunopathology, 112, 210-224.

Toft N, Nielsen SS, Jørgensen E, 2005. Continuous-data diagnostic tests for paratuberculosis as a multi-stage disease. Journal of Dairy Science, 88: 3923-3931.

<u>The following publications have been submitted but are not yet approved</u>

Ersbøll AK, Nielsen LR. Range of spatial correlation between cattle herds of relevance for local spread of *Salmonella* Dublin in Denmark. Submitted to Preventive Veterinary Medicine, January 2007

Lomborg SR, Nielsen LR. Immune Suppression of Cattle Suspected as Carriers of *Salmonella* Dublin. Submitted to BMC Veterinary Research, January 2007.

Nielsen SS, Toft N, Jørgensen E, Bibby BM. Herd-specific sero-prevalences of paratuberculosis in 100 Danish dairy herds using Bayesian mixture models and continuous ELISA response. Submitted to Preventive Veterinary Medicine, Aug. 2006.

Nielsen SS, Toft N. Management related risk factors for paratuberculosis in Danish dairy herds. Submitted to Preventive Veterinary Medicine, Aug. 2006.

CONTRIBUTIONS AT CONFERENCES, SYMPOSIA AND THE LIKE

Baggesen DL, Nielsen LR, Sørensen G, Berrada R, Ersbøll AK. Evaluation of the analytical sensitivity of bacteriological methods for investigation in S. Dublin infected cattle herds. Oral presentation at I3S-International Symposium Salmonella and Salmonellosis, May 10 – 12, 2006, Saint-Malo, France.

Ersbøll AK, Nielsen LR, 2007. Distances between neighbouring herds of relevance for spread of *Salmonella* Dublin between cattle herds in Denmark. Oral presentation at annual meeting of Society of Veterinary Epidemiology and Preventive Medicine, Helsinki, Finland, 28 – 30 March 2007.

Grell SN, Riber U, Clemensen A, Jungersen G, 2004. Induction of apoptosis after specific stimulation with PPDj in *Mycobacterium paratuberculosis* infected cattle. 7th International Veterinary Immunology Symposium, Quebec City, Canada 2004.

Grell SN, Skovgaard K, Clemensen A, Jungersen G. Is antigen specific apoptosis of blood lymphocytes predictive of progressive paratuberculosis? *In* : Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005. p. 151.

Jungersen G, 2003. Status of Immunological Research on Paratuberculosis at Danish Veterinary Institute. Invited talk at Workshop on Paratuberculosis, Feb. 24-27 2003, University of Otago, Dunedin, New Zealand.

Jungersen G, Grell SN, Clemensen A, Howard CJ, 2004. The potentiated interferon-gamma test rescues dayold blood samples for antigen specific cellular mediated immune responses. 7th International Veterinary Immunology Symposium, Quebec City, Canada 2004

Jungersen G, Grell SN, Clemmensen A, Roust T, Howard CJ, 2005. Interleukin-12 potentiation of the interferon-gamma test rescues day-old blood samples for diagnosis of paratuberculosis specific cellular mediated immune responses. Key-note presentation. *In* : Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005, p. 501-506.

Jungersen G. Immunopathogenesis of paratuberculosis. Summary of workshop in: Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005, p. 718-719.

Jungersen, G, 2005. Paratuberculosis, where are we? A short summary of results presented at the 8th International Colloquium on Paratuberculosis. Cattle Consultancy Days, September 1-2, 2005, Nyborg, Denmark.

Kudahl AB, Sorensen JT, Nielsen SS, Ostergaard S, 2006. Perspectives of improved quality of diagnostic tools in paratuberculosis control. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, p. 1001-1003.

Kudahl AB. Economics of paratuberculosis control – from a researchers perspective. Oral presentation at workshop in 8. International Colloquium of Paratuberculosis, Copenhagen August 19. 2005 Summary of workshop in: Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005, p. 720-721.

Kudahl AB. Technical and economical evaluation of strategies against paratuberculosis. Cattle Consultancy Days, Nyborg, September 1-2, 2005

Lomborg SR, Nielsen LR, 2006. Immune Suppression of Cattle Suspected as Carriers of *Salmonella* Dublin. Poster. Poster and proceeding at I3S-International Symposium Salmonella and Salmonellosis, Saint Malo, France. May 10-12, 2006.

Manning EB, Nielsen SS (editors), 2005. Proceedings of the 8th International Colloquium on Paratuberculosis, August 14-18, 2005, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, pp. 734.

Nielsen LR, van den Borne B, van Schaik G, 2006. *Salmonella* Dublin infection in young dairy calves: Transmission parameters estimated from field data and an SIR-model. Proceedings at Annual meeting of Society of Veterinary Epidemiology and Preventive Medicine, March 28-30, 2006.

Nielsen LR, 2004. Intervention at herd level. The use of risk assessment, management strategies and test- and cull procedures to control *Salmonella* Dublin. Cattle Consultancy Days, September 16-17, 2004, Nyborg, Denmark.

Nielsen LR, Ersbøll AK, 2004. Risk factors for becoming a persistent carrier of *Salmonella* Dublin in infected dairy herds. Proceedings of the meeting of the Society of Veterinary Epidemiology and Preventive Medicine (SVEPM), Martigny, Schwitzerland, March 24-26, 2004, pp. 111-123.

Nielsen LR, Nielsen SS, 2006. Risk assessment and management strategies to control *Salmonella* Dublin and paratuberculosis in dairy herds. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, no. 510.

Nielsen LR, Nielsen SS. The use of risk assessment, test and management strategies to control paratuberculosis and *Salmonella* Dublin in dairy herds. *In* : Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005. p. 218-225.

Nielsen LR, Rattenborg E, Nielsen J, 2003. National surveillance program for *Salmonella* Dublin in Danish cattle. Proceedings of the 10th Symposium of the International Symposium of Veterinary Epidemiology and Economics, Nov. 17-21, 2003, Viña del Mar, Chile.

Nielsen LR, Rattenborg E, Nielsen J, 2006. Development of the National Surveillance Programme for *Salmonella* Dublin in Danish cattle. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia: ISVEE 11, no. 870.

Nielsen LR, Toft N, Ersbøll AK, 2003. Comparison of results from a classic and a latent class method for test validation of a *Salmonella* Dublin ELISA. Proceedings of the 10th Symposium of the International Symposium of Veterinary Epidemiology and Economics, Nov. 17-21, 2003, Viña del Mar, Chile.

Nielsen SS, Baptista F, 2007. Operation Paratuberculosis. The Danish control programme for bovine paratuberculosis. Poster presented at annual meeting of Society of Veterinary Epidemiology and Preventive Medicine, Helsinki, Finland, 28 – 30 March 2007.

Nielsen SS, Enevoldsen C, Toft N, 2006. Milk production losses associated with bovine paratuberculosis diagnosed from repeated testing. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, p. 619-621.

Nielsen SS, Houe H, Toft N, 2004. Endemic infectious diseases in the cattle population - eradication, vaccination, control, or do nothing? Proceedings from Cattle Consultancy Days 2004, Nyborg, Denmark, p. 149-154.

Nielsen SS, Jepsen ØR, Aagaard K, 2006. Control programme for paratuberculosis in Denmark. Proceedings of the 1st ParaTB Forum, Shanghai, China, Oct. 19, 2006, p. 21-26.

Nielsen SS, Nielsen LR, 2004. Approaches to *Salmonella* Dublin in Danish cattle. Svenska Djurhälsvårdens fortbindningskonferens, Billighus, Skövde, March 24-25, 2004.
Nielsen SS, Toft N, 2006. Purpose-related interpretation of diagnostic test-information used for bovine paratuberculosis. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, p. 620-622.
Nielsen SS, Toft N, Bibby BM, Nielsen A, 2003. Estimation of sero-prevalence of paratuberculosis with mixture models including covariates. Summary for Proceedings of the 10th International Symposium for Veterinary Epidemiology and Economics, Nov. 17-21, 2003, Viña del Mar, Chile.
Nielsen SS. Control of paratuberculosis in Denmark: considerations on herd and national level. Proceedings from Cattle Consultancy Days, Sept. 1-2, 2005, Nyborg, Denmark, pp. 69-74.
Sergeant ES, Nielsen SS, Toft N, 2007. Estimating freedom of paratuberculosis in Danish dairy herds. Poster presented at annual meeting of Society of Veterinary Epidemiology and Preventive Medicine, Helsinki, Finland, 28 – 30 March 2007.
Skovgaard K, Pudrith CB, Grell SN, Heegaard PMH, Jungersen G, Coussens PM. Differential expression of genes encoding CD30L and P-selectin in cattle with Johne's disease: Progress toward a diagnostic gene expression signature. *In* : Manning EB, and Nielsen SS (eds). Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, August 14-18, 2005. p. 576.
Toft N, Hojsgaard S, Jorgensen E, Nielsen SS, 2006. The effect of correlation structures on the properties of diagnostic tests for paratuberculosis. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 6-11 August 2006. Cairns, Australia, p. 578.
Toft N, Nielsen LR, Højsgaard S, 2003. Exploring disease definitions of *Salmonella* Dublin in latent class analysis. Proceedings of the 10th Symposium of the International Symposium of Veterinary Epidemiology and Economics, Nov. 17-21, 2003, Viña del Mar, Chile.

PROFESSIONAL PUBLICATIONS (most in Danish)

Jungersen G, 2003. Infektionsforløb og immunologisk diagnostik ved paratuberkulose. Dansk Vet.Tidskr. 86. 6-10.
Kudahl AB, 2004. Paratuberkulose kan koste dyrt. Ny Kvægforskning, nr. 2 (2. årg.), p. 2
Kudahl AB, 2004. Økonomi omkring bekæmpelse af paratuberkulose. Ny Kvægforskning, nr. 5 (2. årg.), p. 3
Kudahl AB, 2005. Economic Consequences of Paratuberculosis Control in Dairy Herds. PhD thesis. DIAS report no. 63, June 2005.
Kudahl AB, 2006. Paratuberkulose kan koste dyrt. Bovilogisk, no. 1, 2006, pp. 26-27.
Mortensen H, Nielsen SS, Berg P, Aamand, GP, 2003. Paratuberkulose er arvelig. Ny Kvægforskning, 1 (4), p. 3.
Nielsen LR, 2003. *Salmonella* Dublin hos kvæg på godt og ondt. Oversigtsartikel, KvægInfo nr. 1242. (http://www.lr.dk/kvaeg/informationsserier/lk-meddelelser/1242.htm)
Nielsen LR, 2004. Flere salmonella-infektioner i sensommer og efterår. Månedsmagasinet Kvæg, August.
Nielsen LR, 2005. Praktisk håndtering af Salmonella i malkekvægsbesætninger. Danske Mælkeproducenter, September 2005, pp. 10-13.
Nielsen LR, 2006. Når *Salmonella* smitter dyr og mennesker. Danske Mælkeproducenter, Dec. 2006, pp. 26-27.
Nielsen SS, 2003. Test-strategi i forbindelse med diagnostik af paratuberkulose hos malkekvæg (in Danish). KvægInfo nr. 1201, Dansk Kvæg. http://www.kvaegforskning.dk (http://www.lr.dk/kvaeg/informationsserier/lkmeddelelser/1201.htm)
Nielsen SS, 2004. Diagnostik af paratuberkulose – sikkerhed og usikkerhed i forbindelse med diagnosen hos malkekøer. Danske Mælkeproducenter, 1/2004, 22-24.
Nielsen SS, 2006. Bekæmpelse af paratuberkulose. http://www.paratuberkulose.dk
Nielsen SS, 2006. Betydning af test-resultater fra køer med paratuberkulose. Bovilogisk, januar 2006, p. 24-25.
Nielsen SS, 2006. Effektiv bekæmpelse af paratuberkulose med Bang-metoden. Proceedings of Dansk Kvæg Kongres 2006, p. 94-95.
Nielsen SS, 2006. Generelt om paratuberkulose. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose – statusvurdering i besætningen. Bovilogisk, januar 2006, p. 20-23.
Nielsen SS, 2006. Paratuberkulose: Andre diagnostiske test. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose: Diverse. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose: Forekomst og udbredelse. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose: Forslag til teststrategi. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose: Fortolkning af mælketest. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose: Retslige forhold. http://www.paratuberkulose.dk
Nielsen SS, 2006. Paratuberkulose: Styringslister. http://www.paratuberkulose.dk
Nielsen SS, 2006. Produktionstab som følge af paratuberkulose. http://www.paratuberkulose.dk

Nielsen SS, 2006. Teststrategier og -fortolkning ved sanering af paratuberkulose. http://www.paratuberkulose.dk
Nielsen SS, Nielsen LR, 2004. Indsats mod Paratuberkulose og *Salmonella* Dublin, 3rd ed., pp. 128.
Nielsen SS, Nielsen LR, 2005. Indsats mod Paratuberkulose og *Salmonella* Dublin, (Advisors manual to intervention against paratuberculosis and *Salmonella* Dublin (In Danish)) 4th ed. (2 parts).
Nielsen SS, Nielsen LR, 2006. Håndtering af *Salmonella* Dublin og Paratuberkulose hos ammekvæg. Dansk Kødkvæg, April 2006, p. 12-13.
Nielsen SS, Nielsen LR, 2006. *Salmonella* Dublin og paratuberkulose – kan bekæmpes effektivt. Dansk Kvæg Årsrapport 2005, p. 26-27.
Nielsen SS, Nielsen LR, 2007. Indsats mod Paratuberkulose og *Salmonella* Dublin, 5th ed. (2 parts). (http://www.lr.dk/kvaeg/diverse/netudgave.htm).

RELATED PUBLICATIONS, MASTER THESES ETC.

Berrada RP, 2006. Improved detection of *Salmonella* Dublin in samples from cattle. Thesis Master of Science, University of Copenhagen and Danish Institute for Food and Veterinary Research.
Christensen RB, 2005. Udskillelsesdynamik af *Salmonella* Dublin hos kvæg fra kronisk inficerede besætninger og hos kalve fra udbrudsbesætninger. Veterinary Master thesis. The Royal Veterinary and Agricultural University.
Hansen KR, 2005. Aviditet ELISA til udpegning af potentielle raske smittebærere af *Salmonella* Dublin hos kvæg. Veterinary Master thesis. The Royal Veterinary and Agricultural University.
Lomborg SR, 2006. Immune Suppression of Cattle Suspected as Carriers of *Salmonella* Dublin. Veterinary Master thesis. The Royal Veterinary and Agricultural University.
Nielsen GM, Hansen N, 2007. Effekt af indsats mod *Salmonella* Dublin og høj kalvedødelighed. Veterinary Master thesis. Faculty of Life Sciences, University of Copenhagen.
Nielsen LR, 2003. *Salmonella* Dublin in Dairy Cattle: Use of diagnostic tests for investigation of risk factors and infection dynamics. PhD thesis. The Royal Veterinary and Agricultural University, Denmark. Printed by Samfundslitteratur Grafik, Frederiksberg, Denmark.
Nielsen LR, Ersbøll AK, 2005. Factors associated with variation in bulk-tank milk *Salmonella* Dublin ELISA ODC% in dairy herds. Preventive Veterinary Medicine, 68, 2-4, 165-179.
Nielsen LR, Warnick LD, Greiner M, 2006. Risk Factors for Changing Classification Level in the Danish *Salmonella* Surveillance Program for Dairy Herds. Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics, Cairns, Australia: ISVEE 11, no. 511.
Nielsen LR, Warnick LD, Greiner M, 2007. Risk Factors for Changing Classification Status in the Danish Surveillance Program for *Salmonella* in Dairy Herds. Journal of Dairy Science. Accepted for publication January 2007.
Warnick LD, Nielsen LR, Nielsen J, 2004. Development and estimation of the effect of new methods and strategies for surveillance of salmonella in cattle. Project report. International Epilab, The Institute of Danish Food and Veterinary Research, Søborg, Denmark.
Warnick LD, Nielsen LR, Nielsen J, Greiner, M. 2006. Simulation model estimates of test accuracy and predictive values for the Danish *Salmonella* surveillance program in dairy herds. Preventive Veterinary Medicine, 77, 284-303.
Warnick LW, Nielsen LR, 2004. Results from an EpiLab project: Evaluation of the *Salmonella* Dublin Surveillance Program in Danish Dairy Cattle. Cattle Consultancy Days, September 16-17, 2004, Nyborg, Denmark.
Warnick LW, Nielsen LR, Nielsen J, Matthias Greiner, 2005. Estimating test accuracy and predictive values for the Danish *Salmonella* Dublin surveillance program in dairy herds. Proceedings of the meeting of the Society of Veterinary Epidemiology and Preventive Medicine, Nairn, Scotland, March 30-April 1, 2005.

OTHER COMMUNICATIONS

Proceedings and Closing seminar March 22, 2007 at the National Veterinary Institute, Copenhagen, 48 pp.
Information stand at the annual congress of the Danish Cattle Federation, Herning Kongres Center, February 26-27, 2007
Two midway evaluation meetings for farmers and local veterinarians in the interventions project were held in June 2005 by Søren Saxmose Nielsen, Liza Rosenbaum Nielsen and Gregers Jungersen.
Five two-day courses about practical approaches to controlling paratuberculosis and *Salmonella* Dublin for veterinarians and cattle consultants were held in 2004-2006. Total number of participants approximately 112.
Nordic Veterinary Meeting, Stockholm, Sweden: Seminarium om smittskydds- och djurhälsofrågar för nötkreatur", October 2003.